UNIVERSITY OF KWAZULU-NATAL

THE DISCOVERY AND CHARACTERIZATION OF ANTIPROTOZOAL COMPOUNDS FROM SOUTH AFRICAN MEDICINAL PLANTS BY A HPLC-BASED ACTIVITY PROFILING TECHNIQUE

2013

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TSHOLOFELO ABEDNEGO MOKOKA

2013

A thesis submitted to the School of Chemistry, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville, for the degree of Doctor of Philosophy.

This thesis has been prepared according to **Format 4** as outlined in the guidelines from the Faculty of Science and Agriculture which states:

This is a thesis in which chapters are written as a set of discrete research papers, with an overall introduction and final discussion, where one (or all) of the chapters have been published. Typically, these chapters will have been published in internationally recognized, peer- reviewed journals.

As the candidate's supervisor, I have approved this thesis for submission.

Supervisor:

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ABSTRACT

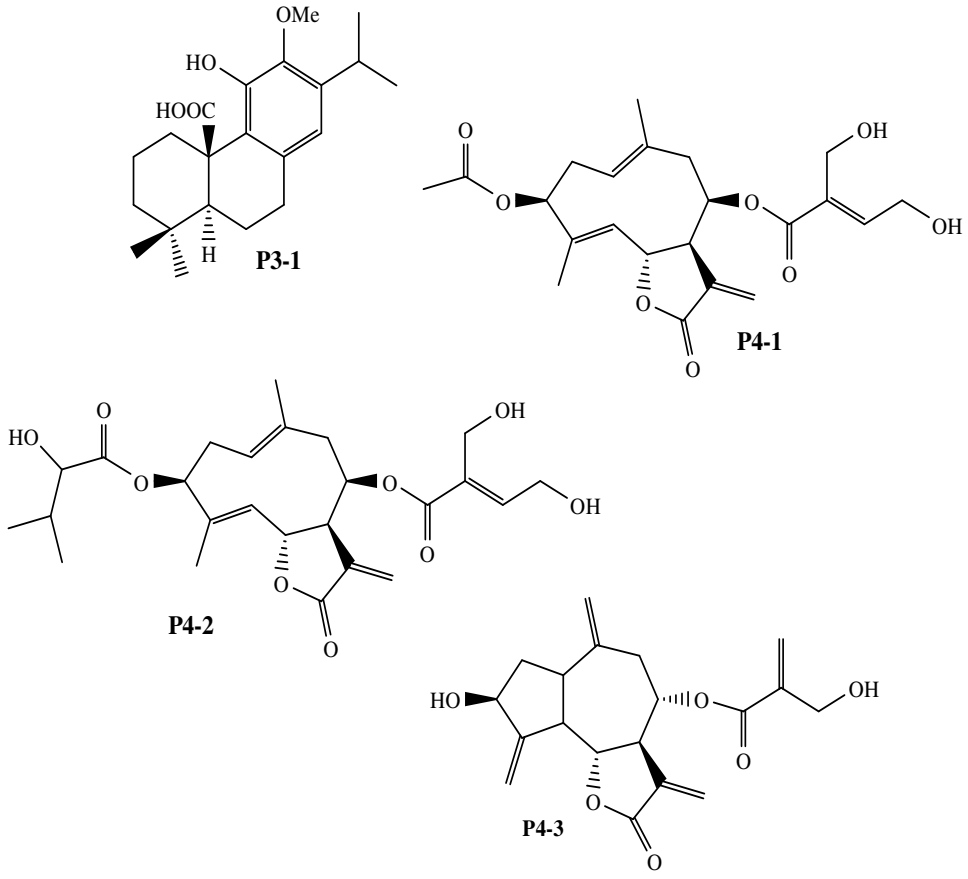
A total of 507 plant extracts from 174 South African plant species, selected based on their traditional uses against parasitic diseases, mainly against malaria were screened against *Plasmodium falciprum, Trypanosoma brucei rhodesiense, Leishmania donovani* and *Trypanosoma cruzi*. One-hundred and seven (107) plant extracts showed more than 95% growth inhibition against one or more parasites and were considered active. The IC_{50} values for these plant extracts in the different assays were determined as well as cytotoxicity evaluations. Plant extracts of *Hypericum aethiopicum* leaves, *Leonotis leonurus* leaves, *Ekebergia capensis* fruits, *Alepidea amatymbica* whole plant, *Asystasia gangetica* leaves, *Setaria megaphylla* whole plant, *Leonotis ocymifolia* var *ocymifolia* leaves, *Schefflera umbellifera*, *Salvia repens* whole plant, and *Tachonanthus camphorates* exhibited IC_{50} values of $\leq$ 5 µg/ml, which was used as second criteria for selecting the plant extracts for phytochemical investigation. The extracts of *Schkuhria pinnata*, *Vernonia mespilifolia* and *Salvia repens* showed promising activity against the parasites of *T. b. rhodesiense*, *P. falciparum* and *L. donovani*. In a medium throughput screening, 64 extracts inhibited the growth of one or more parasites to more than 95% at a concentration of 9.7 µg/ml The most notable results are the antitrypanosomal activities of *S. pinnata* (IC_{50} value of 2.04 µg/ml) and *V. mespilifolia* (1.01 µg/ml). *S. repens* whole plant extract showed anitleishmanial activity with an IC_{50} value of 5.4 µg/ml and cytotoxicity tests against myoblast L6 cells resulted in an IC_{50} of 41.5 µg/ml.

With the aid of a HPLC-based activity profiling technique four compounds (**P3-1, P4-1, P4-2** and **P4-3**) from these three plant species were identified and characterised as the active compounds responsible for the observed activities against the parasites, *T. b. rhodesiesnse*, *P. falciparum* and *L. donovani*. Sesquiterpene lactones, schkuhrin I (**P4-1**) and schkuhrin II

(**P4-2**) from *S. pinnata* and cynaropicrin (**P4-3**) from *V. mespilifolia* were isolated while 12-methoxycarnosic acid (**P3-1**) was isolated from *S. repens*. Cynaropicrin (**P4-3**) was the most active sesquiterpene lactone against *T. b. rhodesiense* (0.23 µM). However, its antiprotozoal activity might be attributed to its toxicity as indicated by cytotoxicity against rat myoblast cells (IC_{50} of 1.29 µM). The sesquiterpene lactones (**P4-1** and **P4-2**) from *S. pinnata* showed promising activity against *T. b. rhodesiense* (IC_{50} values of 0.86 and 1.50 µM). However, compounds **P4-1** and **P4-2** exhibited toxic effects against rat myoblast cells with IC_{50} values of 5.26 and 9.03 µM. Therefore, they are not excellent candidates as lead compounds against *T. b. rhodesiense*. While compounds **P4-1-P4-3** and **P3-1** were shown to be toxic, derivatisation of the compounds, which would change the structure and render them non-toxic could be useful if the derivatised compounds show similar activity against these parasites.

12-Methoxycarnosic acid (**P3-1**) showed antileishmanial activity (IC_{50} of 0.75 µM) and cytotoxicity against L6-cells (IC_{50}, 17.3 µM). The selectivitiy index of 23 indicates that **P3-1** is moderately toxic against the rat myoblast cells. However, it is not a good potential to be considered as a lead drug against leishmaniasis but possible derivatisation of the compound could render it less toxic.

STRUCTURES OF ISOLATED COMPOUNDS



Structures of the isolated compounds schkuhrin I (**P4-1**) and schkuhrin II (**P4-2**) from *Schkuhria. Pinnata*, cynaropicrin (**P4-3**) from *Vernonia mespilifolia* and 12-methoxycarnosic acid (**P3-1**) from *Salvia repens*.

ABBREVIATIONS

VL	Visceral leishmaniasis
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immunodeficiency syndrome
SANBI	South African National Biodiversity Institute
HPLC	High Performance Liquid Chromatography
DMSO	dimethyl sulfoxide
PBS	phosphate buffer solution
FBS	fetal bovine serum
PDA	Photo Diode Array
NMR	Nuclear Magnetic Resonance
MS	Mass Spectrometry
SI	selectivity index
DHT	dihydrotestosterone
AR	androgen receptor
SM	simple monophasic
^{13}C NMR	C-13 nuclear magnetic resonance spectroscopy
^{1}H NMR	proton nuclear magnetic resonance spectroscopy
$CDCl_3$	deuterated chloroform
TLC	thin layer chromatography
UV	ultraviolet
DALYs	disability adjusted life years
CSIR	Council for Scientific and Industrial Reserach
NTDs	neglected tropical diseases
HAT	human African trypanosomiasis
SSA	sub-Saharan Africa

WHO	World Health Organization
DRC	Democratic Republic of Congo
BBB	blood-brain barrier
CNS	central nervous system
DFMO	DL-α-difluoromethylornithine
LDL	low density lipoprotein
CL	cutaneous leishmaniasis
MCL	mucocutaneous leishmaniasis
OIs	opportunistic infections
Sb^{v}	pentavalaent antimonials
Sb^{III}	trivalent antimonials
CDC	center for disease control and prevention
DEC	disease endemic countries
MMV	Medicine for Malaria Venture
STPH	Swiss Tropical and Public Health
DCM	dichloromethane
MeOH	methanol

DECLARATIONS

DECLARATION 1 – PLAGIARISM

I, TSHOLOFELO ABEDNEGO MOKOKA declare that

1. The research reported in this thesis is my original research, except where otherwise indicated.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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Signed ……

DECLARATION 2-PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part of and/or include research presented in this thesis.

Publication 1

Mokoka T. A., Zimmermann, S., Julianti, T., Hata, Y., Moodley, N., Cal, M., Adams, M., Kaiser, M., Brun, R., Koorbanally, N., Hamburger, M., 2011. *In vitro* screening of traditional South African Malaria remedies against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. Planta Medica 77, 1663-1667.

Publication 2

Tsholofelo A. Mokoka, Xolani. K. Peter, Gerda Fouche, Nivan Moodley, Michael Adams, Matthias Hamburger, Marcel Kaiser, Reto Brun, Vinesh Maharaj, Neil Koorbanally. Antileishmanial activity of 12-methoxycarnosic acid from *Salvia repens* Burch. ex Benth. (Lamiaceae). South African Journal of Botany *in press*.

Publication 3

Tsholofelo A. Mokoka, Xolani. K. Peter, Gerda Fouche, Stefanie Zimmermann, Nivan Moodley, Michael Adams, Marcel Kaiser, Vinesh Mahara, Neil A. Koorbanally, Matthias Hamburger, Reto Brun, 2013. Antiprotozoal screening of 60 South African plants and the identification of the antitrypanosomal germacranolides schkuhrin **I** and **II**. Planta Medica 79, 1380-1384.

In all the publications above, my role included carrying out all the experimental work and contributing to the writing of the publications along with my supervisors. The other co-authors contribution was that of an editorial nature and checking on the scientific content and my correct interpretation. Based on their expertise, they have added minor parts to the manuscripts. This project was collaboration between the University of KwaZulu-Natal, the University of Basel, Switzerland and the CSIR (Pretoria, South Africa). Members from each of the institutions contributed to the manuscripts and are listed as authors.

Signed: ……..

ACKNOWLEDGEMENTS

I would like to say thank you to the almighty GOD for giving me life, strength and courage to be able to work and finish this project.

I would also like to send my whole hearted appreciations to Dr Gerda Fouche for her scientific guidance, encouragement, humility, emotional support and for believing in me throughout the duration of this project.

Dr Vinesh Maharaj for providing me with an opportunity to work and complete this project at the Council for Scientific and Industrial Research (CSIR), Natural Products Chemistry Laboratories.

Dr Neil Anthony Koorbanally for his effortless encouragement, guidance and scientific input in compiling this thesis.

Thank you to Dr Xolani Kevin Peter for teaching me to run the NMR and for the interpretation of spectra for structure elucidation. You played a tremendous role in the completion of this project.

Dr Paul Steenkamp for running the mass spectra of the isolated compounds and helping with the interpretation thereafter.

Dr Nivan Moodley for his scientific input and guidance when called upon.

Many thanks also go to Dr Michael Adams and Professor Matthias Hamburger (both from the University of Basel, Switzerland) for teaching me the HPLC-based activity profiling technique and the scientific editing of the three papers forming this thesis.

I would also like to say thank you to Dr Reto Brun and Marcel Kaiser (both from the University of Basel, Switzerland) for effortlessly performing the antiprotozoal screening assays.

Acknowledgements to my family Marius Mokoka (father), Eunice Mokoka (mother), Moditsaotsile Mokoka, Robert Mokoka, Lemogang Mokoka, Rodney Mokoka, Thato

Mokoka, Sello Mokoka and Reuben Mokoka, thanks for encouraging and supporting me to finish my studies.

My friend, Obokeng Letseka you are the best for all the encouragements that I got from you.

My appreciations also go to Gugulethu Ndlovu, Itumeleng Setshedi, Jeremia Senabe, Rudzani Nthambeleni, Schalk van Rooyen, Oscar Marumong, Andries Seolwane and Felicia Mbombela with whom we shared jokes, frustrations and even encouraged each other throughout the years, guys you are my other family.

To my girlfriend, Tshegofatso Molefe thanks for the understanding, encouragements and for your support during my study.

I would like to acknowledge the Department of Science and Technology (DST), Council for Scientific and Industsrial Research (CSIR) and the Swiss Federation for providing financial support for this project and myself.

THIS IS DEDICATED TO MY GRANDMOTHER, SINAH MASTEMBERE MOKOKA.

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CHAPTER 1

Introduction

Infectious and parasitic diseases pose a global problem to the human population. The neglected tropical diseases (NTDs) are a subset of infectious diseases caused by 13 parasitic and bacterial infections[1, 2] and include three vector-borne protozoan infections (human African trypanosomiasis, Leishmaniasis and Chagas disease), three bacterial infections (trachoma, leprosy and Buruli ulcer) and seven helminth infections (hookworm, ascariasis, trichuriasis, lymphatic filariasis, onchocerciasis, drancunculiasis and schistosomiasis) [3]. The parasitic and bacterial infections responsible for these diseases are listed in table 1.1 [3]. NTDs mostly occur in rural and impoverished urban areas of low-income countries. They have the deleterious effect of impairing childhood growth, intellectual development, education and worker productivity [4]. The cause of these diseases are partly attributed to inadequate access to clean water, poor sanitation, and shack dwelling and apart from disfiguring patients there is also a stigma attached to the diseases [2, 4].

NTDs cause an estimated 534 000 deaths annually [4], which place a large burden on healthcare demands and economic development of low-income countries. In addition, approximately 57 million disability-adjusted life years (DALYs) are lost due to premature disability and death per year [5], an even greater burden than those for malaria and tuberculosis [6]. NTDs represent the fourth most important group of communicable diseases worldwide behind lower respiratory infections, Human Immunodeficiency virus/Acquired immunodeficiency syndrome (HIV/AIDS) and diarrheal diseases [4] and are the second only to HIV/AIDS as a cause of disease burden [3].

Table 3.1 The 13 parasitic and bacterial infections responsible for neglected tropical diseases (NTDs) around the world [3]

Etiological agent	Parasitic diseases
Protozoan	
Leishmania donovani	Visceral leishmaniasis (Kala-azar)
Trypanosoma gambiense and *Trypanosoma brucei rhodesiense*	African trypanosomiasis (African sleeping sickness)
Trypanosoma cruzi	American trypanosomiasis (Chagas disease)
Helminth	
Schistosoma mansoni, S. haematobium, S. japonicum	Schistosomiasis
Wuchereria bancrofti and *Brugia malayi*	Lymphatic filariasis (elephantiasis)
Onchocerca volvulus	Onchocerciasis (river blindness)
Dracunculiasis medinensis	Drancunculiasis (guinea worm)
Ascaris lumbricoides	Ascariasis (roundworm)
Trichuris trichiura	Trichuriasis (whipworm)
Necator americanus and *Ancylostoma duodenale*	Hookworm
Bacterial	
Mycobacterium leprae	Leprosy
Mycobacterium ulcerans	Buruli ulcer
Chlamydia trachomitis	Trachoma

In September 2000, world leaders at the United Nations adopted the Millennium Declaration, which established eight millennium development goals to eliminate extreme poverty, hunger

and disease by 2015. The sixth goal specifically focuses on health and economic impact of infectious diseases by combating malaria, tuberculosis and HIV/AIDS. This goal has led to considerable and welcomed large scale financial injection through initiatives sponsored by the group of eight (G8) governments to fight HIV/AIDS and malaria [1]. However, until recently, programs to combat other diseases like NTDs have not benefited from these support systems. For the scope of this project, only three vector-borne protozoan infections (*Trypanosoma brucei rhodesiense, Leishmania donovani* and *Trypanosoma cruzi*) and malaria (*Plasmodium falciparum*) are investigated.

1.1 Malaria

Malaria is one of the most dangerous parasitic infections with severe public health and development challenges worldwide, mainly in the tropical and subtropical areas [7]. It is a mosquito-borne disease caused by four protozoan parasites of the genus *Plasmodium* (*P. vivax, P. ovale, P. malariae*and *P. falciparum*) that infect the red blood cells. *P. falciparum* is responsible for a large proportion of morbidity and most malaria deaths in endemic regions [8]. It is transmitted to people through the bites of infected *Anopheles* mosquitoes, called "malaria vectors"and is characterized by symptoms of fever, headache, chills and vomiting which appear seven days or more after infection [9]. If left untreated within 24 hours, *P. falciparum* malaria can progress to severe illness often leading to death. In children, severe anaemia, respiratory distress in relation to metabolic acidosis and cerebral malaria may frequently develop [10].

In 2010, an estimated 216 million cases of malaria were reported per annum with an estimated 655 000 deaths with 86% of these deaths being children [9, 11]. This indicated a marginal reduction in the number of deaths (863 000) reported in 2008 [12]. In sub-Saharan

Africa, *P. falciparum* accounts for approximately 90% of the cases and almost all malaria deaths each year, mainly in African children under the age of 5 years and in pregnant woman [9]. Economically, malaria slows the economic growth by 13% per year which translates into a gross domestic product cost in sub-Saharan Africa of US$ 12 billion per year, causing severe economic consequences to the poor areas of the sub-Saharan region [13], preventing improvement and advancement in both the economy and living standards and places serious constraints on the country's ability to fund and maintain programmes that could control malaria [8].

1.1.1 Malaria and HIV/AIDS co-infections

In 2011, an estimated 2.5 million people were newly infected with HIV resulting in over 34.2 million people living with HIV globally, and over 1.7 million deaths annually. An estimated 23.5 million of the total HIV infected population were residing in the sub-Saharan African region alone with 1.7 million infections being new cases. The annual mortality rate arising from the disease in sub-Saharan Africa is over 1.2 million people [14]. Malaria, reported as one of the leading causes of HIV-related morbidity in sub-Saharan Africa [16] is responsible for an estimated 91% deaths in sub-Saharan Africa [9, 11]. There is considerable geographical overlap between the two diseases with the most severely affected areas being Zambia, Zimbabwe, Mozambique, Malawi and the Central African Republic [15].

HIV has been shown to increase the risk of malaria infection and the development of clinical malaria, with the greatest impact in immune-suppressed individuals [17-19]. Conversely, malaria has been shown to increase the viral load by inducing HIV-1 replication both *in vitro* and *in vivo* [20, 21]. In 2004, Grimwade and co- investigators conducted a study in Hlabisa district, KwaZulu-Natal, South Africa in a total of 1109 malaria antigen-positive patients

[18]. In this study 613 patients were confirmed microscopically to have been suffering from malaria. In addition, 180 (29.4%) of the 613 microscopically confirmed patients were HIV positive and 152 (30.6%) of the 496 unconfirmed patients were also HIV positive resulting in the total number of 332 (29.9%) HIV patients co-infected with malaria. The results suggested that HIV infection increases the risk of severe malaria and subsequently also increases the risk of death [18].

In a similar study conducted by Cohen and colleagues at Chris Hani Baragwanath hospital, Johannesburg, South Africa, 336 patients were enrolled in the study to assess the impact of HIV infection on the risk of severe malaria [22]. In this study, 110 (33%) patients were HIV positive and 111 (33%) patients were non-immune (born or residing in an area without stable malaria transmission) and 225 (68%) patients were semi-immune (born and spent at least 5 years of childhood in an area with high levels of malaria transmission). HIV-infected patients in the non-immune group showed greater incidences of severe malaria than the semi-immune group [22]. This suggested that HIV infection is associated with an increased risk of severe malaria, probably because of an impaired immune response to the malaria parasite leading to a decreased ability to control parasitemia. In addition, severe malaria was more common in patients with a $CD4^+$ T cell count $<200 \times 10^6$ cells/L [22].

1.1.2 Current antimalarial drugs and resistance development

Malaria is one of the most infectious diseases in the world and the emergence of resistance to available drugs is a major contributing factor to most of malaria-associated deaths in endemic regions [23, 24]. The discovery of quinine (**1**), the first antimalarial drug in the 17^{th} century from the bark of *Cinchona calisaya* and the subsequent discovery of artemisinin (**2**) – also known as Qinghaosu - in the 1970s from a Chinese medicinal plant, *Artemisia annua*

commonly known as “qinghao”, contributed significantly to the treatment and eradication of malaria for many years [25]. Currently, several classes of antimalarials (quinoline derivatives, antifolates, artemisinin derivatives and antimicrobials) have been developed and are used for the treatment and prevention of malaria infection [26-28].

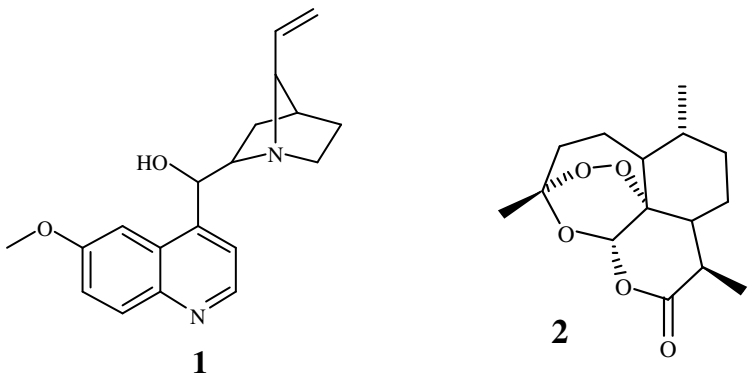

Figure 4.1 Structures of some of the currently used antimalarial agents, quinine (**1**) and artemisinin **(2)**

Besides being used to combat malaria, currently used antimalarials also have certain liabilities like toxicity, poor compliance, rapid resistance development and are extremely costly in developing countries. The synthetic derivatives of quinine (**1**) such as 8-aminoquinoline (primaquine) and 4-aminoquinoline (chloroquine) have played a significant role in the control and eradication of malaria in endemic regions. In addition, the synthesis of chloroquine-like drugs led to the discovery of lumefantrine, piperaquine and pyronanidine in China and amodiaquine, mefloquine and halofantrine in the United States [29]. These quinine drugs cause parasite death by blocking the polymerization of the toxic by-products of haemoglobin degradation into insoluble and non-toxic pigment granules, resulting in cell lysis and parasite cell autodigestion [30].

1.2 Human African Trypanosomiasis

Human African trypanosomiasis (HAT) also known as sleeping sickness is a disease caused by protozoan parasites of the genus *Trypanosoma*, single-celled organisms that remain in extracellular form in the host. There are two forms of the human disease, the East and Southern African variant caused by *Trypanosoma brucei rhodesiense* and the West African form caused by *Trypanosoma brucei gambiense*. This disease is transmitted in both humans and cattle by the bite of a blood-sucking tsetse fly of the *Glossina* species. Animals are the main host for the *T. b. rhodesiense* parasite while humans host the *T. b. gambiense* parasites. HAT is a major threat to the health of 60 million people living in 36 countries in the sub-Saharan African (SSA) region and is the world's third most important parasitic disease affecting human health after malaria and schistosomiasis, as defined by the global burden of parasitic diseases, calculated as the disability adjusted life years (DALYs) lost. This disease is very fatal if left untreated resulting in high mortality rates in endemic regions [31].

In the 1960s, HAT caused by *T. b. gambiense* was controlled in Central Africa as a result of effective surveillance programs but it quickly re-emerged with a progressive increase in the numbers of new cases and deaths [32]. In the last decade, the number of new cases reported per annum in major endemic countries in SSA has decreased considerably as a result of increased public health. In 2005, an estimated 50 000-70 000 cases of HAT were reported in SSA with only 17 616 cases reported as new but this number has been significantly reduced in recent years [31, 32]. Recently, the World health organization (WHO) reported an estimated 30 000 cases of HAT in endemic regions and only 7139 new cases per annum. Most of the new cases of HAT were reported in the Democratic Republic of Congo (DRC) with over 500 new cases. Some of the countries included Angola, Central African Republic, Chad, Sudan, and Uganda which reported between 100 and 500 new cases per year [31].

In the host, the parasite (*T. b. rhodesiense* or *T. b. gambiense*) invades the lymph nodes and systemic organs such as the liver, spleen, heart, eyes and endocrine systems immediately after one to three weeks of infection. This stage is termed the hemolymphatic stage (stage 1) and if left untreated for a few weeks (*T. b. rhodesiense*) or many months (*T. b. gambiense*), the parasites cross the blood-brain barrier (BBB) and enters the central nervous system (CNS). This stage is termed the encephalitic stage (stage 2) of the disease [31]. In the hemolymphatic stage, HAT is characterised by nonspecific symptoms such as weight loss, headache, malaise, arthralgia, fatigue, fever and vomiting [31]. The encephalitic stage is characterised by sleeping disturbances, psychiatric and mental features, motor and sensory disturbances and visual involvement [33].

1.2.1 Human African Trypanosomiasis and HIV/AIDS co-infections

Human African Trypanosomiasis (HAT) and HIV/AIDS are endemic in most of the sub-Saharan countries making co-infections very common, which poses a major socio-economic and public health problem in these countries. The impacts of HIV/AIDS on the epidemiology and/or clinical manifestation of HAT are still very unclear. However, few studies have shown that HIV-infected patients might be at a higher risk of treatment failure and unfavourable outcomes [34, 35]. Recent reports have shown a few cases of HIV and HAT co-infection [36, 37].

1.2.2 Current antitrypanosomal drugs and resistance development

Currently, four drugs discovered decades ago are used in the treatment of HAT [38]. In addition, these drugs have unacceptable toxicity, an undesirable route of administration, is very expensive and not effective at all stages of the disease [38, 39]. Three of the drugs,

suramin (**3**), pentamidine (**4**) and melasoprol (**5**) were developed during the first half of the twentieth century and the last drug to be registered for HAT, DL-α-difluoromethylornithine (DFMO) (**6**) was registered in 1990 [31, 33, 38]. In the hemolymphatic stage, suramin (**3**) and pentamidine (**4**), discovered in 1921 and 1941, respectively are used as the drugs of choice against *T. b. rhodesiense* and *T. b. gambiense*, respectively because they are not able to cross the BBB [31, 33, 39]. Suramin (**3**) can also be used as an alternative therapy against *T. b. gambiense* [33].

In the encephalitic stage, melasoprol (**5**), discovered in 1949 is used against both *T. b. rhodesiense* and *T. b. gambiense*. However, DL-α-difluoromethylornithine (**6**) is used against *T. b. gambiense* only [31, 33]. Recent developments have led to the use of nifurtomix (**8**) - DL-α-difluoromethylornithine (**6**) combination as an alternative treatment for *T. b. gambiense* in the encephalitic stage [31, 33] and is provided free of charge by the WHO [31]. The following paragraphs will provide an in depth knowledge about the possible mode of administration, mode of action, adverse effects and resistance development associated with each drug.

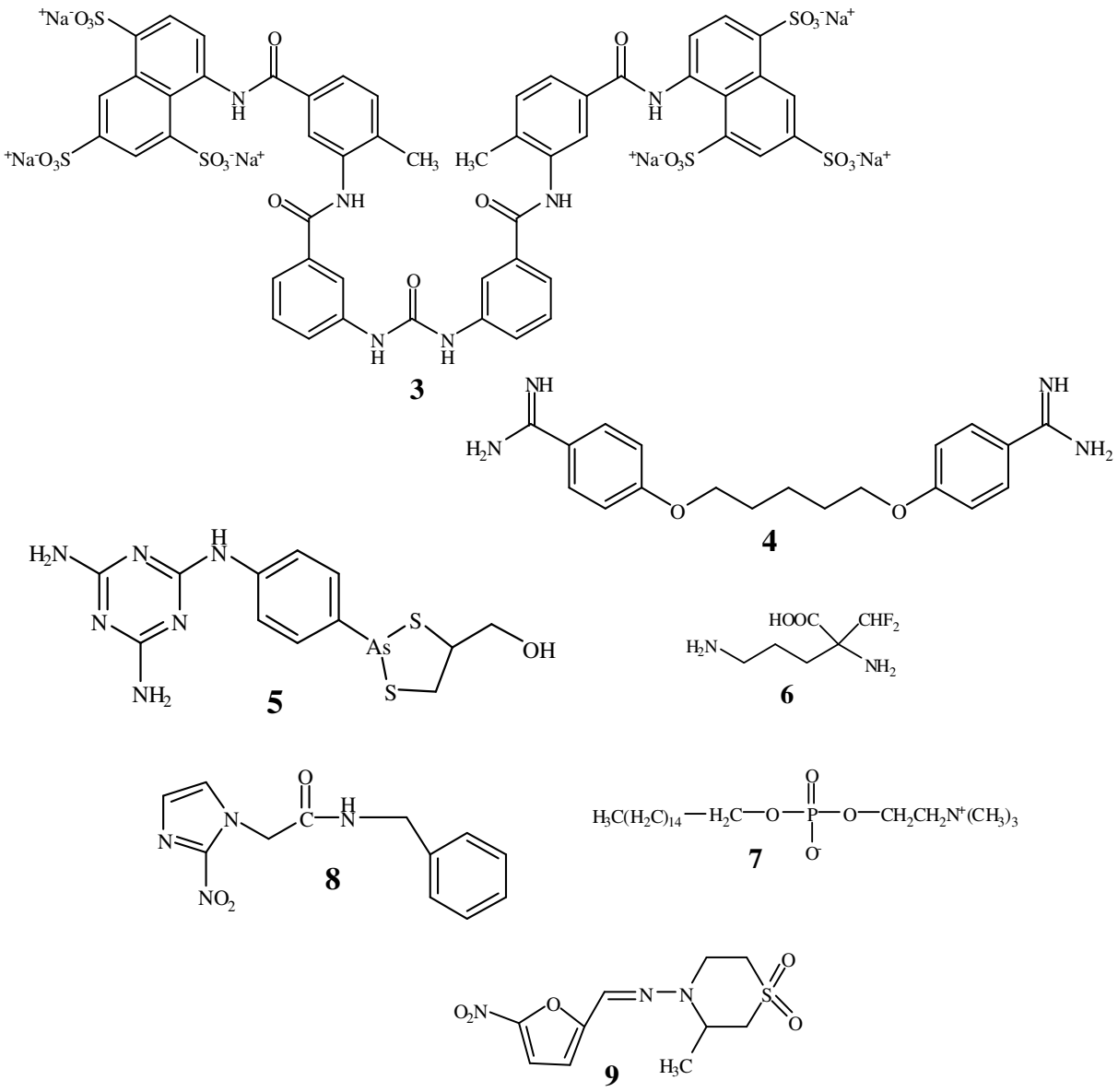

Figure 1.5 Structures of some of the currently used antiprotozoal drugs [Suramin (**3**), Pentamidine (**4**), Melasoprol (**5**), DL-α-difluoromethylornithine (**6**), Benznidazole (**7**), Nifurtimox (**8**) and Miltefosine (**9**)] against *T. b. rhodesiense*, *L. donovani* and *T. cruzi*

Suramin (**3**) is a colourless, polyanionic sulfonated napthylamine that is highly soluble in water and has to be given intravenously by injection [38]. Some of the immediate adverse effects include collapse with nausea, vomiting and shock. Severe delayed reactions include kidney damage in malnourished patients, exfoliate dermatitis, agranulocytosis, haemolytic

anaemia, jaundice and severe diarrhoea, all of which can be fatal [38]. Unfortunately, suramin (**3**) does not penetrate well into the CNS and is therefore only effective in the hemolymphatic stage [38]. The mode of action of suramin in killing trypanosomes is still not known and its action is very slow [38]. It has been suggested that the action of suramin (**3**) involves inhibition of various glycolytic enzymes such as reverse transcriptase, dihydrofolate reductase, fumarase, glycerol-3-phosphate dehydrogenase, hexokinase, L-α-glycerophosphate oxidase, receptor mediated uptake of low-density lipoprotein (LDL), RNA polymerase and kinases, thymidine kinases and trypsin [40, 41, 42]. Vansterkenburg and co-authors suggested that deprivation of the parasite from cholesterol and phospholipids by an inhibition of the uptake of LDL contributes to the mode of action of suramin (**3**) against trypanosomes [43]. No significant clinical resistance against suramin (**3**) has emerged despite its use for many decades [38].

Pentamidine (**4**), an aromatic diamidine remains the drug of choice for early *T. b. gambiense* infections [44]. Unfortunately, pentamidine (**4**) has poor oral bioavailability because it is highly protonated at physiological pH, hence it is given intramuscularly for a week [38, 44]. Generally, pentamidine (**4**) is well tolerated but when given by intramuscular injection, site pain and transient swelling, abdominal pain, gastrointestinal problems, hypoglycaemia, hypocalcemia and renal failure are frequently reported [41, 44]. The mode of action of pentamidine (**4**) has not been established [38, 45]. However, its toxic effect could arise from the inhibition of multiple cellular targets [45]. Vercesi and Docampo suggested that, pentamidine (**4**) decreases the mitochondrial membrane potential of trypanosomatids [46] and act as an uncoupler of oxidative phosphorylation in mammalian mitochondria [47]. It also selectively inhibits the plasma membrane Ca^{2+} ATPase in *T. b. brucei* [48]. Resistance to pentamidine (**4**) has been induced in laboratory models [42].

Melasoprol (**5**), an organoarsenic compound, remains the most widely used drug for the treatment of encephalitic stage HAT caused by both *T. b. gambiense* and *T. b. rhodesiense* in resource-poor countries where DL-α-difluoromethylornithine (**6**) is not available or affordable [38, 44]. Melasoprol (**5**) is practically insoluble in water and must be given intravenously dissolved in propylene glycol [38]. Adverse reactions are frequent and can be severe or even life-threatening. The most important reaction is an encephalopathic syndrome which occurs in 5-10% of the cases [41, 44]. Other common side effects include vomiting, abdominal colic, peripheral neuropathy, arthralgia and thrombophlebitis [38]. Due to these adverse effects particularly, encephalopathy, safer alternatives to melasoprol (**5**) are needed urgently. Treatment failures have occurred in 30% of those treated, suggesting the emergence of resistance to melasoprol [44].

DL-α-difluoromethylornithine (**6**) is the only drug that has been recently registered for the treatment of human African trypanosomiasis [31, 33, 38, 44] recently. It is an analogue of the anticancer agent ornithine [45]. DL-α-difluoromethylornithine (**6**) is recommended as the first-line treatment for second stage *T. b. gambiense* infections [31, 33, 38] and is administered by continuous infusion because of the short half-life of the drug [44]. It has a slow action and is trypanostatic rather than trypanocidal [41]. It is also poorly absorbed and rapidly excreted in the urine [42]. Adverse drug reactions include bone marrow toxicity leading to anaemia, leucopenia and thrombocytopenia, gastrointestinal symptoms and convulsions [49]. Its mode of action is through the suicide inhibition of ornithine decarboxylase, the key enzyme in the pathway leading to the biosynthesis of polyamines: putrescine, spermidine and spermine which are essential for cell proliferation [45]. No drug

resistance has been reported in the field even though some drug-resistant mutants have been isolated in the laboratory [45].

The drugs mentioned above have significantly contributed to the eradication and treatment of human African trypanosomiasis in poor countries. However their associated toxicities, mode of administration and all the other challenges necessitates an urgent need in the discovery and development of novel drugs which are active at all stages of the disease, which is cheap, non-toxic and can be easily administered in a short course.

1.3 Leishmaniasis

Leishmaniasis comprises a group of tropical diseases caused by twenty species of protozoan parasites belonging to the genus *Leishmania*. These parasites cause one of four clinical forms of the diseases, cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), diffuse cutaneous leishmaniasis and visceral leishmaniasis (VL) [50]. The disease affects approximately 12 million people worldwide with around 2 million new cases reported annually in 88 countries. A population of over 350 million individuals are at risk of infection in the tropic and subtropic regions of the world [51, 52]. Leishmaniasis is transmitted zoonotically by the female flying insects of the genus *Phlebotomus* sandflies in Africa, Asia and Europe and *Lutzomyia* in the Americas and Australasia [53].

Visceral leishmaniasis (VL) caused by *Leishmania donovani* is the most severe form of leishmaniasis. It is associated with high mortality and morbidity rates in developing countries and remote areas around the world and can be very fatal if left untreated [54]. This agent affects around half a million people annually mainly in India, Nepal, Bangladesh, Sudan, Ethiopia, and Latin American countries such as Brazil [50, 53, 54]. In sub-Saharan

Africa, most visceral leishmaniasis cases are reported in the Horn of Africa, i.e., the east African countries of Sudan, Eritrea, Ethiopia, Kenya and Somalia [55]. The highest incidence of the disease occurs in Sudan where 15000-20000 new cases are reported annually, followed by Ethiopia with approximately 4000 new cases reported per annum [55, 56]. Many cases occur in areas of conflict which has destroyed housing and health care infrastructure. Forced human migrations make the disease burden estimates difficult. Increased malnutrition as a result of drought and human conflict contributed severely to the emergence and progression of visceral leishmaniasis in endemic countries [57].

Visceral leishmaniasis is characterised by fever, weight loss, hypergammaglobulinemia, pancytopenia and affects the internal organs, mainly the liver and spleen [58, 59]. These conditions are very fatal if treatment is not established in time.

1.3.1 Leishmaniasisand HIV/AIDS co-infections

Opportunistic infections (OIs) are one of the leading causes that result in a decline in the condition of HIV-infected patients. Of these, parasites play an important role as OIs and are one of the most common causes of morbidity and mortality in HIV/AIDS patients [61]. Leishmaniasis is one of the two main systematic OIs reported in HIV-infected patients [61]. It attacks the patient's immune system, already compromised by HIV/AIDS, making the situation worse and the patient more susceptible to other infections such as tuberculosis and pneumonia. The impact of the HIV/AIDS pandemic has modified the natural history of leishmaniasis. HIV immunosuppression increases the risk of acquiring leishmaniasis by 100-1000 times in endemic regions and encourages the development of relapsing and eventually drug resistant leishmaniasis. The possible overlap of these two diseases is therefore very dangerous [63]. Recent increases in the prevalence of VL-HIV co-infections in Southern

Europe, South Asia, Brazil and sub-Saharan Africa has created interesting diagnostics and therapeutic problems.

1.3.2 Current antileishmanial drugs

Current treatment of leishmaniasis is primarily based on chemotherapy and to some extent immunotherapy [64] since there is no vaccine against this disease [65]. For many years, pentavalent antimonials (Sb^{v}) such as sodium stibogluconate and meglumine antimoniate, introduced more than 50 years ago have been used as drugs of choice for the treatment of leishmaniasis [54]. The mechanism of action of these drugs is still unknown, but it is reported that their antileishmanial action is probably due to the *in vivo* reduction of the Sb^{v} form to a more toxic trivalent antimonial (Sb^{III}) form. Generally, it is known that Sb^{v} acts by inhibiting glucolysis, fatty acid beta-oxidation, ADP phosphorylation [66, 67] and DNA topoisomerase in the parasite [68]. It also alters the thiol-redox potential of the parasite by actively promoting efflux of the thiols, glutathione and trypanothione, thus rendering the parasite more susceptible to oxidative stress [69].

One main advantage of the antimonials is their low cost but adverse effects including cardiac arrhythmias, elevated hepatic transaminase blood levels, pancreatitis and pneumonitis [70, 71]. The emergence of resistance against the pentavalent antimonials in the state of Bihar, India constituted a major problem in the treatment of visceral leishmaniasis. This resistance was primarily acquired and driven mainly by inadequate treatment and poor compliance with these drugs [72]. Therefore, their administration has been abandoned in this area and replaced by the conventional amphotericin B, amacrolidepolyene antifungal antibiotic agent that has been used as a second-line treatment for visceral leishmaniasis since the 1960s. This drug exhibits an excellent antileishmanial activity with more than 90-95% success rate in

India [73]. The introduction of amphotericin B lipid formulations has improved the safety profile of this drug. Lipid formulations of amphotericin B: liposomal amphotericin B, amphotericin B lipid complex and amphotericin B cholesterol dispersion are taken selectively by the reticulo-endothelial system and exhibit a highly localized enhanced antileishmanial activity [74]. Frequent adverse effects related to amphotericin B treatment; include infusion-related fever and chills, nephrotoxicity and hypokalemia [75].

Another current antileishmanial drug, paromomycin is an aminoglycosidic aminocyclitol isolated in 1956 from the *Streptomyces riomosus* var. *paromomycinus*. Its antileishmanial activity has been reported in the 1960s by Neal [76]. Paromomycin has shown a 94.6% cure rate of visceral leishmaniasis in India in the phase III clinical trials [77]. Paromomycin inhibits protein synthesis and modifies membrane fluidity and permeability [74]. Paromomycin related adverse effects include elevated hepatic transaminases, ototoxicity and pain at the site of injection [77].

An exciting new development in the treatment of visceral leishmaniasis has been the discovery of miltefosine (**9**), an alkylphospholipid, in the 1980s as a novel drug for the treatment of visceral leishmaniasis that can be administered orally in endemic countries [54]. Based on its safety and efficacy profile, miltefosine (**9**) was registered in India in 2002 and subsequently in Germany in 2004 [78]. In a phase IV multicenter clinical trial, miltefosine (**9**) exhibited a cure rate of 82% per intention-to-treat analysis and 95% per protocol analysis in 1132 Indian visceral leishmaniasis patients [79]. In this study, adverse effects associated with miltefosine (**9**) treatment such as reversible gastrointestinal and renal toxicity, elevated hepatic transaminases and creatinine [79] were reported. The major drawbacks of miltefosine (**9**) are the potential of teratogenicity and its long half-life (approximately 150 hours) which

may promote the emergence of resistance, endangering the life span of miltefosine (**9**) [59, 74]. Miltefosine (**9**) is also forbidden in women of child-bearing age who may become pregnant up to two months of discontinuing the drug [80, 74].

Generally, many of the current antileishmanial drugs have disadvantages including the intramuscular route of administration and prolonged hospitalization and duration of treatment [70, 71]. Some drugs are very expensive as is the case with amphotericin B and its lipid formulations [73, 77] and their use is limited due to lack of efficacy [81, 82]. Emergence of resistance parasites and toxicity of these drugs is still a major concern in the treatment of visceral leishmaniasis [77].

1.4 Chagas disease

Chagas disease, also known as American trypanosomiasis is caused by the protozoan parasite, *Trypanosoma cruzi* and was discovered in 1909 by the Brazilian physician Carlos Ribeiro Justiniano Chagas (1879-1934) [83, 84]. This disease is transmitted to humans and domestic and wild animals such as dogs, cats, guineapigs, rodents, armadillos and marsupials by large blood sucking insects of the subfamily Triatominae [85]. Three triatomine species, *Triatoma infestans*, *Rhodnius prolixus* and *T. dimidiata* are the most important vectors in the transmission of the parasite to humans [86, 87]. Commonly, infection is acquired through contact with the faeces of an infected triatomineor "kissing bug" while feeding on the host. The infected faecal droplets may be inadvertently be passed to the mucosa of the eye, nose or mouth causing infection to the host [88]. It can be transmitted through several mechanisms by blood transfusions, from mother to child (congenital transmission), through contaminated food like meat, sugar cane juice and fruit juice and accidental contamination during laboratory work [83, 84, 88].

The disease is endemic in Latin American regions from northern Mexico to Argentina and some cases have been reported in the USA and Canada [83, 89, 90]. The WHO reported that an estimated 10 million people are infected with *T. cruzi* worldwide, mostly in Latin America with over 25 million people living at risk [83]. In the acute phase, Chagas disease infection is characterised by skin chancre (chagoma) or unilateral purplish orbital oedema (Romaña sign) with local lymphoadenopathies, and this can be accompanied by fever, headaches, abdominal pains, hepatomegaly, splenomegaly, myocarditis (chest pain and heart failure), and more rarely meningoencephalitis, especially in AIDS patients [83, 84]. Death occurs occasionally in the acute phase especially to children under the age of five [83, 84]. In the chronic phase, this disease hides in the heart and digestive smooth muscles and is characterised by arrhythmia, cardiomyopathy, heart failure, secondary thromboembolism, some digestive lesions such as megaoesophagus and megacolon and eventually death [83, 91-93].

1.4.1 Chagas disease and HIV/AIDS co-infections

Due to the mass migration of rural inhabitants to big cities and an increase in poverty, this has led to co-infection of the disease with HIV and is a well-known opportunistic infection in people living with HIV/AIDS [94, 95].

Several non-endemic countries like Australia, Canada, Japan, Spain and the United States have observed an increased public health impact due to the overlap of HIV/AIDS infection and *T. Cruzi* [96]. In 2012, there were 74 deaths in which Chagas disease and HIV/AIDS were mentioned as the cause of death in which 57 (77%) was due to AIDS as the underlying cause and 13 (17.6%) being attributed to Chagas disease [96]. The most important clinical consequence to Chagas disease and HIV/AIDS co-infection is the reactivation of latent

(chronic) *T. cruzi* infection due to a suppressed immune system [94, 97]. Co-infection may also emerge as acute Chagas disease among those with AIDS living in Chagas endemic areas and acquiring recent *T. cruzi* infection [98]. This reactivation of Chagas disease may affect the central nervous system (CNS) with expansion lesions or meningoencephalitis and cardiopathy [99].

1.4.2 Treatment of Chagas disease and resistance development

There is no vaccine for Chagas disease. Currently, benznidazole (**7**) and nifurtimox (**8**) are used in the treatment of Chagas disease, which are completely effective when administered just after infection [83]. However, the efficacy of these drugs diminishes the longer a person has been infected [83]. Both drugs are not approved for use in the USA but can be obtained from the Center for Disease Control and Prevention (CDC) and used under investigational protocols [100].

Benznidazole (**7**) and nifurtimox (**8**) are nitroheterocyclic drugs that have been used for the treatment of Chagas disease for over forty years [101]. Generally, both drugs act through the formation of free radicals and/or electrophilic metabolites. The nitro group of both drugs is reduced to an amino group by the action of nitroreductases, with the formation of various free radical intermediates and electrophilic metabolites. These free radicals bind to lipids, proteins and DNA, damaging them [102]. The trypanocidal activity of benznidazole (**7**) does not entirely depend on free radical formation. It has been shown that benznidazole (**7**) improves phagocytosis, increases trypanosomal death through IFN-γ [103], and inhibits *T. cruzi* NADH-fumarate reductase [104]. Some of the adverse effects associated with these drugs include systemic toxicity, anorexia, nausea, vomiting, headache, central nervous

system depression or maniacal symptoms, seizures, vertigo, paresthesias, peripheral polyneuropathy and dermatitis [105].

1.5 Natural products as antiparasitic agents

The search for a new alternative treatment for protozoal diseases among plants has developed momentum in the drug discovery and development programs for several years [106, 107]. This search for better and less toxic novel substances derived from natural products has been based on the ethno-medicinal use of the plants by the indigenous population in endemic areas. Several reviews have listed plants and natural product derivatives that showed some level of promising antiparasitic activity [108, 109]. Plant extracts and natural compounds from these medicinal plants have shown very promising antiprotozoal activities and most importantly provide leads toward the discovery of novel molecules for the treatment of these diseases. The following paragraphs will provide a rationale and reflect some of the research done over the years in the discovery of active antiprotozoal compounds from nature with particular emphasis on malaria, leishmaniasis, sleeping sickness and Chagas disease.

1.5.1 Natural products with antileishmanial activity

A review by Chan-Bacab and Peña-Rodríguez mentioned approximately 97 natural compounds that have shown very promising antileishmanial activity [108]. The most promising classes were terpenes: diterpenoids [110] and sesquiterpenes [111], phenolics, naphthoquinones [112, 113] and alkaloids [114]. The structures of some of the natural agents that have shown promising antiprotozoal activity are shown in **Figure 1.3**. Among these is berberine (**10**), a quaternary isoquinolinic alkaloid with leishmanicidal activity found in plant families such as Annonaceae, Berberidaceae and Menispermaceae. Plants from these families have been used in various folk remedies for the treatment of cutaneous

leishmaniasis, malaria and amoebiasis [115]. This metabolite has been used clinically for the treatment of leishmaniasis for over 50 years and it has been demonstrated that it possesses significant activity both *in vitro* and *in vivo* against several species of *Leishmania* [116].

Plumbagin (**11**), a naphthoquinone isolated from species of the genus *Plumbago* has shown antileishmanicidal activity with IC_{50} values of 0.42 and 1.1 µg/ml against *L. donovani* and *L. amazonensis*, respectively [117]. Diospyrin (**12**), a bis-naphthoquinone isolated from the bark of *Diospyros montana* (Ebenaceae) is reported to be active against promastigotes of *L. donovani* with a MIC of 1.0 µg/ml [118]. The oxygenated chalcone, licochalcone A (**13**) isolated from the roots of the Chinese licorice plant (*Glycyrrhiza spp.*) inhibits the *in vitro* growth of promastigotes of *L. major* and *L. Donovani* [119, 120]. Triterpenes such as ursolic acid (**14**) and betulinaldehyde (**15**) from the bark of *Jacaranda copaia* and the stem of *Doliocarpus dentatus* respectively have shown activity against the amastigotes of *L. amazonensis* [121, 122]. Even though some of the reported natural products have been used for centuries in folk medicine, none have been evaluated in clinical studies. This might be attributed to the lack of promising activity when tested *in vivo* and also to the toxic effects of some of the compounds in experimental assays.

1.5.2 Natural Products with antitrypanosomal activity

The discovery of active antiprotozoal molecules from medicinal plants has been well documented. Recent reviews by Uchiyama [123] and Izumi [124] have reported the antitrypanosomal activity of a wide variety of medicinal plant extracts and their constituents against *T. cruzi*. Sixty-one compounds belonging to flavonoids, phenylpropanoids, monoterpenes, sesquiterpenes and diterpenes and 134 compounds isolated from plants have been reported as showing potent trypanocidal activity [123].

The sesquiterpene lactone, helenalin (**16**), isolated from *Helenium autumnale* exhibited an IC_{50} value of 0.695 µM against *T. cruzi* [125]. Although many compounds have shown antitrypanosomal activity, to date none have been registered for clinical use, probably due to poor results when tested *in vivo*, unacceptable toxicity results in experimental assays and lower potency compared to the currently used drugs, benznidazole and nifurtimox [126].

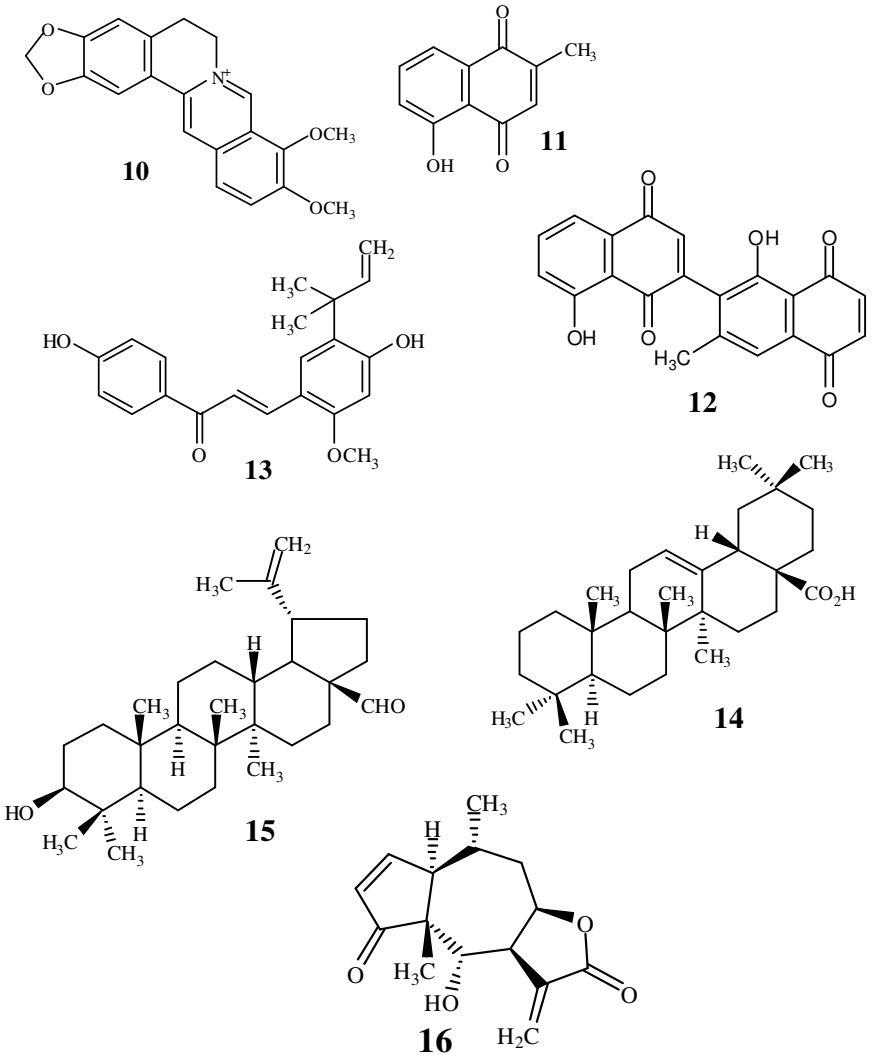

Figure 1.6 Structures of some of the natural products [Berberine (**10**), Plumbagin (**11**), Diospyrin (**12**), Licochalcone A (**13**), Ursolic acid (**14**), Betulinaldehyde (**15**) and Helenalin (**16**)] with promising antiprotozoal activity isolated from medicinal plants

Several reviews have reported many medicinal plants being used in the treatment of human African trypanosomiasis (HAT) commonly known as sleeping sickness, most of which are used on the African continent where sleeping sickness is endemic [127, 128, 129]. There have been reports of a wide variety of medicinal plants being tested for antipyranosomal activity in Uganda, Côte d'Ivoire, Benin and Sudan [127-130], the most being recorded in Côte d'Ivoire with 88 plants being tested against *T. b. rhodesiense* [128]. The most active plant extracts were the roots of *Albizia gummifera* ($IC_{50}$of 70 ng/ml), *Enantia polycarpa* (IC_{50} of 0.5 µg/ml), *Trichilia emetica* (IC_{50} of 0.04 µg/ml) and *Xanthium brasilicum* (IC_{50} of 0.1 µg/ml) against *T. b. rhodesiense* [127-129]. A subsequent bioassay-guided isolation of *Xanthium brasilicum* resulted in the identification of xanthanolides as the active compounds [129].

1.5.3 Natural Products as antimalarials

In South Africa, medicinal plants are commonly used in healthcare to treat a range of ailments, which include malaria and its associated symptoms [131]. There are several reports of medicinal plants being used traditionally to treat malaria in South Africa [132, 133], Kenya [134], Zimbabwe [135] Congo [136], Brazil [137], Nigeria [138, 139] and Cameroon [140]. The many medicinal plants used in the healthcare system are indicative of the crucial role that medicinal plants play in many developing countries. These plants are also sources of novel plant-derived compounds which could be leads for pharmaceuticals against several diseases.

Two of the currently used antimalarial drugs, quinine and artemisinin have originated from plant sources. In the last ten years, five reviews have appeared with over 200 compounds isolated from medicinal plants showing antiplasmodial activity [141-145]. These compounds

were also in diverse classes of compounds such as alkaloids, quassinoids, terpenes, sesquiterpenes, triterpenoids, chalcones, flavonoids, xanthones, quinones, coumarins, limonoids and peptides. The indole alkaloids were found to exhibit IC_{50} values under the micromolar range and with good selectivity index [143].

1.6 HPLC based activity profiling in drug discovery

Many of the active compounds in plants have been discovered by the use of an essential technique, bioassay-guided fractionation. However, in recent years the use of this technique in drug discovery has resulted in a number of setbacks, especially being criticised for finding already known active metabolites. In addition, this technique is time consuming, tedious and not suited for a medium throughput setting [146]. The introduction of hyphenated analytical techniques such as LC/DAD-UV, LC/MS, GC/MS and LC/NMR in which a chromatographic separation is coupled online with one or more information rich detectors have revolutionised the discovery of novel molecules from natural products [147]. One important advantage of these techniques is the identification of known molecules at an early stage of discovery. Hence, the isolation of unknown active compounds is prioritised.

Fractionation of complex mixtures such as medicinal plant extracts by High Performance Liquid Chromatography (HPLC) in deep-well microtiter plates in combination with a biological activity assay is extensively employed to unambiguously identify bioactive compounds [148]. Recently, several bioactive compounds have been isolated and identified by using a HPLC-based activity profiling technique [149-152]. This technique has been used extensively in recent times in the discovery of antiprotozoal agents from natural plants extracts [153-156]. Generally, in this approach sub-milligram amounts of extract are separated by analytical scale HPLC and automatically fractionated into 96 well plates. The

microfractions obtained are submitted to the bioassay, and the resulting activity profile can be overlaid with the HPLC trace to correlate peaks of activity with peaks in the HPLC chromatogram. On-line spectroscopic data (UV–Vis and MS) collected during separation, combined with database searches provide structural information on the active principles [146, 153, 157] making the identification of known active metabolites possible at an early stage of discovery rather than wasting time in the isolation process of known molecules.

1.7 Aim of the project

The aim of this project was to use South African plants that have been preselected on the basis of indigenous knowledge for the discovery of molecules with *in vitro* activity against *Trypanosoma brucei rhodesiense*, *Leishmania donovani*, *Plasmodium falciparum* and *Trypanosoma cruzi* that causes neglected diseases. The discovery of such molecules will ultimately lead to the development of new and improved pharmacotherapy for these diseases, and to a reduction in the number of fatalities and disabilities associated with these diseases in poor and disease endemic countries (DEC).

To achieve this, a library of South African plant species with potential antiprotozoal activity would be identified and from this we would choose the most appropriate plants to work on phytochemically. For the isolation of active compounds from some of the selected plant species, a relatively new technique, High Performance Liquid Chromatography (HPLC)-based activity profiling was used to identify and isolate active compounds from relatively small amounts of plant material. Using this technique, a small quantity (350 µg) of plant extract was fractionated into 96 well microtiter plates and subjected to assay screening, at the same time obtaining the MS and UV data of the extract followed by extensive off-line

analysis to identify the active molecule. This technique is very usefull in the early identification of known plant constituents without isolating them.

1.8 The objectives of the project

The objectives of the project were as follows

1. Screening of a focused library of preselected plants extracts in a medium throughput screening to identify a specific library of plant extracts with antiprotozoal activity.
2. Selection of potential "hits" to be subjected to the HPLC-based activity profiling technique.
3. HPLC semi-preparative isolation of active compounds from potential "hits".
4. Structure elucidation and characterization of the isolated active compounds.
5. *In vitro* biological activity evaluation of the isolated compounds to determine efficacy and toxicity.

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CHAPTER 2

IN VITRO SCREENING OF TRADITIONAL SOUTH AFRICAN MALARIA REMEDIES AGAINST *TRYPANOSOMA BRUCEI RHODESIENSE, TRYPANOSOMA CRUZI, LEISHMANIA DONOVANI* AND *PLASMODIUM FALCIPARUM*

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Abstract

Three hundred extracts were prepared from plants traditionally used in South Africa to treat malaria and were screened *in vitro* for activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum.* For the 43 extracts which inhibited the growth of one or more parasites to more than 95% at 9.7 µg/mL, the IC_{50} values against all four protozoal parasites and cytotoxic IC_{50} values against rat myoblast L6 cells were determined. Amongst the most notable results are the activities of *Agathosma apiculata* (IC_{50} of 0.3 µg/mL) against *Plasmodium falciparum* and *Salvia repens* and *Maytenus undata* against *Leishmania donovani* with IC_{50} values of 5.4 µg/mL and 5.6 µg/mL, respectively. This screening is the starting point for a HPLC based activity profiling project in antiprotozoal lead discovery.

Keywords

South Africa, antiprotozoal, *T. b rhodesiense; T. cruzi; L. donovani*; *P. falciparum*

Introduction

Half a billion people get infected with malaria every year, and 1-2 million die of the disease [1]. Up to thirty million people annually contract one of the so called "neglected tropical diseases", Chagas disease, human African trypanosomiasis or leishmaniasis, and the three diseases lead to 120 000 deaths annually [2]. These diseases are all caused by protozoal parasites, are transmitted by insect vectors, and affect the poorest populations. Malaria drugs are reasonably affordable, available and safe, yet few in number and increasingly compromised by resistances [3]. Only a few drugs are on the market to treat trypanosomatid infections (*Trypanosoma* and *Leishmania*), and their pharmacological profiles are insufficient by modern standards [1]. For decades, large pharmaceutical companies have been reluctant

to invest in the development of new drugs for economic reasons. This may be changing though because there have been increasing drug discovery and development efforts in recent years from private initiatives and non-profit organizations. The Medicines for Malaria Venture (MMV), for instance, has initialized research networks [4], and philanthropist organizations like the Bill and Melinda Gates foundation and others have contributed substantial assets to such endeavors [5].

The goal of this study was to evaluate the antiprotozoal potential of plants used by traditional communities in South Africa to treat malaria. Extracts were screened against the most important protozoal parasites, *Plasmodium falciparum* (malaria), *Trypanosoma cruzi* (Chagas disease), *Trypanosoma brucei rhodesiense* (sleeping sickness) and *Leishmania donovani* (leishmaniasis). This screening study forms the base for an ongoing HPLC based activity profiling project in antiprotozoal lead discovery. This library had been previously screened for antiplasmodial activity against chloroquine sensitive *Plasmodium falciparum* D10 strain [6].

Material and methods

Plant species selection

Plants were collected from the wild at various locations in South Africa by different ethnobotanists based on their ethnomedicinal uses against parasitic diseases, mainly malaria. A list of these uses can be found in an earlier publication [6]. Voucher specimens were identified and deposited at the South African National Biodiversity Institute (SANBI) (Table 2.1, Appendix 2.1, Page 89-108).

Preparation of plant extracts

The plant parts (roots, leaves, twigs, fruits and stem bark) were dried in an oven at 30 – 60 °C. Dried plant material was ground to a coarse powder using a hammer mill and stored at ambient temperature prior to extraction. Of each sample, 100–500 g of powdered material was sequentially extracted, typically with 1 L of cold dichloromethane (DCM), DCM/Methanol (MeOH) (1/1), MeOH, and purified water in 2 L glass jars with screw on lids. The extracts were filtered; the plant material dried overnight in a fume hood and then extracted with the next more polar solvent. Organic extracts were concentrated using a Buchi rotavapor (Essen, Germany) at a temperature below 45 °C and then further dried *in vacuo* at ambient temperature for 24 h. The aqueous extracts were concentrated by freeze-drying. The yields of the extracts, in terms of starting plant material, were recorded. All dried extracts were stored at −20 °C. Analytical grade solvents for extraction were purchased from Romil Pure Chemistry (Cambridge, UK). HPLC grade water was obtained from a TKA Ultra Pure water purification system (Niederelbert, Germany).

Evaluation of *in vitro* antiprotozoal activity

Screening of the extract library against *Plasmodium falciparum* (K1 strain), *Trypanosoma brucei rhodesiense* (STIB 900 strain), *Trypanosoma cruzi* trypomastigote forms (Tulahuen strain), and *Leishmania donovani* (strain MHOM/ET/67/L82) was performed in 96 well plates at concentrations of 9.7 and 1.8 µg/mL. Tests were done in duplicate and repeated twice. IC_{50} values against the parasites as well as cytotoxic effects against rat myoblast (L6-cells) were determined by serial dilution and repeated twice.

Trypanosoma brucei rhodesiense (STIB 900) were grown in axenic medium as described by Baltz et al. [7]. The samples were tested using the Alamar Blue assay protocol [8] to

determine the 50% inhibitory concentration (IC_{50}). A Spectramax Gemini XS micro plate fluorescence reader (Molecular Devices Cooperation, Sunnyvale, CA) with excitation wavelength 536 nm and an emission wavelength 588 nm was used to measure the plates. Melarsoprol (Arsobal®, Sanofi-Aventis, Switzerland) was used as a reference drug (IC_{50}= 0.03 ± 0.01 µM). The IC_{50} values were calculated by using Softmax Pro software (Molecular Devices Cooperation, Sunnyvale, CA).

Trypanosoma cruzi trypomastigote forms (Tulahuen strain C2C4 containing β-galactosidase (Lac Z) gene) were cultured as described by Buckner et al. [9] in rat myoblast cells (L6-cells). Benznidazole (Sigma-Aldrich, Switzerland) was used as a standard drug (IC_{50}= 0.48 µg/mL). After incubation, the substrate chlorophenyl red β-D-galactopyranoside agent CPRG/Nonident was added to all wells and a color change was developed within 2 to 6 h. The plates were read photometrically at 540 nm (Molecular Devices). Data were evaluated and IC_{50} values calculated using Softmax Pro software (Molecular Devices).

Axenically grown *L. donovani* amastigotes (strain MHOM/ET/67/L82) were grown and tests were done as previously described using the resazurin assay [10]. The plates were developed for 2-4 hours and read on a Spectramax Gemini XS micro plate fluorometer (Molecular Devices Cooperation) using an excitation wavelength of 536 nm and emission wavelength of 588 nm. Fluorescence development was measured and expressed as percentage of the negative control. Miltifosine (VWR, Paisley, Scotland) was used as a reference drug (IC_{50}= 0.241 µg/mL). Data were transferred to the graphic programme Softmax Pro (Molecular Devices), with which IC_{50} values were calculated.

Screening of the extract library and determination of activity against *Plasmodium falciparum* K1 strain was done using a modified version of the ^{3}H-hypoxanthine incorporation assay by Trager and Jensen [11]. After incubation the plates were harvested using a Betaplate cell harvester (Wallac, Switzerland) onto glass-fiber filters and washed. The dried filters were inserted into plastic foils with 10 ml scintillation fluid. The radioactivity was counted with a Betaplate liquid scintillation counter (Wallac, Switzerland) as counts per minute per well at each drug concentration and compared to the untreated controls. Chloroquine was used as a positive control (IC_{50}= 0.05 ± 0.01 µM). IC_{50} values were calculated by linear interpolation. All assays were run in duplicate and repeated twice.

The cytotoxicity assay was performed by a similar protocol as the Alamar Blue assay whereby L6-cells were seeded in 100 µl RPMI 1640 supplemented in 96-well micro titer plates (4000 cells/well). Podophyllotoxin (Sigma-Aldrich, Switzerland) was used as the reference drug. After 68 h of incubation under humidified 5% CO_2 atmosphere, 10 µl of the Alamar blue marker was added to all wells. The plates were incubated for an additional 2 h. A Spectramax Gemini XS micro plate fluorescence reader (Molecular Devices Cooperation, Sunnyvale, CA) was used to measure the plates (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values were calculated by Softmax Pro software (Molecular Devices Cooperation, Sunnyvale, CA).

Results and Discusion

One hundred and seven medicinal plants were selected because they had a reported tradition of being used as antiparasitic remedies. Dried plant parts were extracted using solvents of different polarity (DCM, DCM/MeOH (1:1), MeOH and water) to give 300 extracts [6]. In a first step the extracts were screened for their potential anti-parasitic properties against *T. b.*

rhodesiense, *T. cruzi*, *L. donovani* and *P. falciparum* at the test concentrations 9.7 and 1.6 µg/mL (Appendix 1, Table **2.1**, Page 89 - 108).

In a second stage, extracts which in the first step had shown more than 95 % inhibition of one or more parasites at a test concentration of 9.7 µg/ml were selected as potential "hits" and their IC_{50} values against all four parasites as well as their cytotoxicity against rat myoblast cells (Appendix 1, Table **2.2**, Page 109 - 111) were determined. This enabled the direct comparison of activities, parasite specific actions and toxicities of each of our "hits", and served as a basis for the selection of the most promising extracts for HPLC based activity profiling and identification of the active principles [10].

In general, *P. falciparum* was the most sensitive protozoal parasite towards the extract library. The most potent extract in the entire screen was the DCM/MeOH (1:1) extract of *Agathosma apiculata* (Rutaceae) with an IC_{50} of 0.21 µg/mL against *P. falciparum.* Seven more lipophilic (DCM/MeOH (1:1)) extracts showed antiplasmodial IC_{50} values of under 5 µg/mL: that of *Hypericum aethiopicum* leaves (IC_{50}=2.4 µg/mL), *Leonotis leonurus* leaves (2.9 µg/mL), *Ekebergia capensis* fruits (3.5 µg/mL), *Alepidea amatymbica* whole plant (3.7 µg/mL), *Asystasia gangetica* leaves (4.2 µg/mL), *Setaria megaphylla* whole plant (4.4 µg/mL) and *Leonotis ocymifolia* var *ocymifolia* leaves (4.5 µg/mL). Also, the DCM extract of *Leonotis ocymifolia* var *ocymifolia* leaves was very active (2.7 µg/mL) (Appendix 1, Table **2.2**, Page 109 - 111).

Against *Trypanosoma brucei rhodesiense* the DCM/MeOH (1:1) extracts of *Tarchonardus camphorates* whole plant (3.9 µg/mL), *Hypericum aethiopicum* leaves (4.7 µg/mL), *Leonotis ocymifolia* var *ocymifolia* leaves (9.1 µg/mL), *Croton menyhartii* whole plant (8.8 µg/mL)

and the DCM extracts of *Pentzia globosa* roots (5.8 µg/mL), *Vernonia oligocephala* leaves (4.7 µg/mL), *Artemisia afra* leaves (9.6 µg/mL), and *Leonotis ocymifolia* var *ocymifolia* leaves (9.7 µg/mL) showed IC_{50} values lower than 10 µg/mL. Three further DCM, eighteen DCM/MeOH extracts, and one MeOH extract had IC_{50} values between 10 and 20 µg/mL against *Trypanosoma brucei* (Appendix 1, Table **2.2**, Page 109 - 111). Despite the fact that most of the 43 "selected hits" showed relatively good activity against *T. brucei*, none showed selectivity towards this parasite, as it had been the case for the *Plasmodium falciparum* screen.

Trypanosoma cruzi was the least sensitive protozoal test organism. Three extracts, the DCM/MeOH extract of *Hypericum aethiopicum* leaves (18.1 µg/mL), the DCM extract of *Catha edulis* roots (19.1 µg/mL) and the DCM/MeOH extract of *Tarchonardus camphoratus* whole plant (18.6 µg/mL) inhibited *T. cruzi* with IC_{50} values under 20 µg/mL. Seven extracts showed IC_{50} values of less than six µg/mL against *Leishmania donovani*. These were the DCM/MeOH extract of *Hypericum aethiopicum* leaves (4.7 µg/mL), *Leonotis leonurus* leaves (4.7 µg/mL), *Ekebergia capensis* fruits (4.8 µg/mL), *Schefflera umbellifera* (5.0 µg/mL), *Salvia repens* (5.4 µg/mL), *Tachonanthus camphorates* (4.9 µg/mL), and the DCM extract of *Maytenus undata* (5.6 µg/mL).

We screened an extract library generated from plants selected for their traditional antimalarial use against the most important human protozoal parasites. The goal was to identify extracts with potent and, ideally, selective activity against any one of the parasites. These hits are being followed up in an ongoing HPLC-based activity profiling project in antiprotozoal lead discovery.

The initial screening of this focused extract library delivered forty three "potential hits". These were defined as samples with almost complete (> 95%) inhibition at 9.7 µg/mL. Twelve of them showed activity against *Plasmodium falciparum*, twenty eight against *Trypanosoma brucei rhodesiense*. Six of these extracts were active against both parasites. Against *Leishmania donovani*, eleven extracts fulfilled the criteria for "potential hits". Five of these were also active against *T. brucei*. One extract, the DCM extract of *Catha edulis* roots, inhibited both *P. falciparum* and *Leishmania,* and the DCM/MeOH extract of *Hypericum aethiopicum* leaves inhibited the growth of all three parasites. No extract showed > 95 % inhibition of *Trypanosoma cruzi*at 9.7 µg/mL. This parasite is generally less sensitive than *Trypanosoma brucei* because it is an intracellular test system.

In a second phase, the IC_{50} values of the 43 "potential hits" were determined against all the parasites and against L6 cells (Appendix 1, Table **2.1**, Page 89 - 108). *Agathosma apiculata* (Rutaceae) was exceptional in the sense that it was by far the most active extract against *Plasmodium falciparum* and showed at the same time good selectivity. An IC_{50} of 0.3 µg/mL against *Plasmodium* compared to 43 µg/mL in L6 cells corresponded to a selectivity value of 143. The extract was also 140 times more active against *P. falciparum* than against *T. cruzi*, 53 times more active than against *Leishmania donovani*, and 37 times more active than against *T. brucei*. Nine of the 43 selected active extracts showed IC_{50} values of <10 µg/mL against *Trypanosoma brucei rhodesiense* (Appendix **1**, Table **2.1**, Page 89 - 108). Yet, despite the relatively high activity, none showed selectivity towards this parasite.

Salvia repens and *Maytenus undata* were shown to be preferentially active against *Leishmania donovani,* whilst showing only moderate cytotoxic effects (Appendix 1, Table **2.1**, Page 89 - 108). The most active extracts against *T. cruzi* showed nonspecific effects.

The DCM/MeOH extracts of *Hypericum aethiopicum* leaves (18.1 µg/mL) and *Tarchonardus camphoratus* (18.6 µg/mL), and the DCM extract of *Catha edulis* roots (19.1 µg/mL) at the same time strongly inhibited L6 cells, which are their *T. cruzi*'s host cells (Appendix 1, Table **2.1**, Page 89 - 108). The DCM/MeOH and/or DCM extracts of *Hypericum aethiopicum, Leonotis leonurus, Ekebergia capensis, Catha edulis, Asystasia gangetica, Schefflera umbellifera* and *Tarchonardus camphoratus* all showed relatively high cytotoxicity, with IC_{50} values < 20 µg/mL, and activities against most protozoal test organisms. Therefore, these extracts were not shortlisted for further follow-up.

Conclusions

In summary, screening of our focused library led to a shortlist of extracts with high activity against *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense* and *Leishmania donovani* in a primary screen. The percents of inhibition at the two initial test concentrations were roughly indicative of the half maximum inhibition concentration (IC_{50} values) to be expected in the second stage of the screening. Given the cellular test systems and their inherent variability, some extracts showed IC_{50} values slightly higher or lower than could have been predicted from the primary screen. For direct comparison of activities it is therefore necessary to determine IC_{50} values by serial dilution (Appendix 1, Table **2.2**, Page 109 - 111). This data, together with cytotoxicity data, helps to determine whether an activity is specific or just a generally toxic effect. Extracts with favorable activities are followed up by HPLC-based activity profiling [12]. We have recently developed and validated a microtiter-based protocol for the miniaturized and efficient identification of antiprotozoal compounds in extracts [10], and successfully applied this approach to the discovery of new antiprotozoal natural products in other library-based discovery projects [13-15]. Results from the profiling

and identification of the active principles from the extracts in this screen will be reported in due course.

Acknowledgements

The authors would like to thank the Council of Scientific and Industrial Research (CSIR), and the Swiss Confederation for financial support under the Swiss South African Joint Research Programme (grant JRP 03), and SANBI for the identification of plant species.

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CHAPTER 3

ANTILEISHMANIAL ACTIVITY OF 12-METHOXYCARNOSIC ACID FROM *SALVIA REPENS* BURCH. EX. BENTH. (LAMIACEAE)

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Abstract

In South Africa, *Salvia repens* is used traditionaly to treat sores, stomach ache and diarrhoea. The high performance liquid chromatography (HPLC)-based activity profiling of *S. repens* whole plant extract showed an active abietane diterpene, identified as 12-methoxycarnosic acid (**P3-1**) which showed antiprotozoal activity against axenically grown *L. donovani* amastigotes with an IC_{50} of 0.75 µM with marginal cytotoxicity against the L6-cells (IC_{50}, 17.3 µM).

Keywords: Antileishmanial activity; *Salvia repens*; *Leishmania donovani*; 12-methoxycarnosic acid; abietane diterpene; HPLC profiling

Abbreviations: VL = Visceral leishmaniasis; HIV = Human Immunodeficiency virus; SANBI = South African National Biodiversity Institute; HPLC = high performance liquid chromatography; DMSO =dimethyl sulfoxide; PBS = phosphate buffer solution; FBS = fetal bovine serum; PDA =Photo Diode Array; NMR = Nuclear Magnetic Resonance; MS = Mass Spectrometry; SI = selectivity index; DHT = dihydrotestosterone; AR = androgen receptor; SM = simple monophasic.

Introduction

Visceral leishmaniasis (VL), also known as "Kala-azar" is caused by the intracellular protozoan *Leishmania donovanii* and is transmitted by the bite of infected phlebotomine sandflies. It has been estimated that the annual global burden of VL is 500 000 new cases with more than 59 000 associated deaths [1]. The control of VL relies entirely on chemotherapy due to the lack of antileishmanial vaccines. In poor countries, however, the use of leishmanicidal drugs is

hampered by high costs, toxicity and the emergence of resistance [2]. In addition, the emergence of the Human Immunodeficiency virus/Acquired Immunodeficiency disease (HIV/AIDS) has severely compromised the control of VL [3]. Therefore, there is an urgent need for the development of new leishmanial therapeutics.

The genus *Salvia* (sage) is the largest in the Lamiaceae family with over 900 species worldwide [4]. Approximately 30 *Salvia* species occur in southern Africa of which 26 are found in South Africa especially in the Cape region [5]. *Salvia* species are used in folk medicine for the treatment of asthma, eczema, psoriasis and tuberculosis [6]. In South Africa, *Salvia* species are used against fever, headache and digestive disorders [7], and to treat sores [8]. A decoction of the roots is also used in South Africa for the treatment of stomach ache and diarrhoea [8]. Several kinds of tanshinone-type diterpenoids [9] abietane and icetexane diterpenoids and triterpenoic acids [4,10,11], phenolic acids, phenolic glycosides, flavonoids and anthocyanins [12] have been reported from *Salvia* species. These constituents have shown various biological properties including antiviral [13], antiprotozoal [9,14,15] antimalarial [16] and antileishmanial [4] activities.

In our previous study, the dichloromethane/methanol (1:1) extract of the whole plant of *Salvia repens* showed promising antiprotozoal activity with a IC_{50} value of 5.4 µg/ml against axenically grown *Leishmania donovani* amastigotes (MHOM/ET/67/L82) [17]. In this paper we report the isolation and identification of the active component and its antileishmanial activity.

Materials and methods

Plant collection

Salvia repens whole plant material was collected from the Eastern Cape Province near Aliwal-North in January 2002. The identification of plant material was done at the South African National Biodiversity Institute (SANBI, Pretoria) where thevoucher specimen (BP00998) was deposited. The wet plant material was placed in an oven at 40 °C for three days to dry, then milled to a fine powder and kept at room temperature in the dark before extraction.

Extract preparation

Finely ground plant material of *S. repens* (100 g) was extracted with (2 × 1L) of a mixture of dichloromethane and methanol (1:1, v/v) overnight with occasional aggitation. The mixture was filtered and concentrated using a rotavapour at 40 °C and left under a stream of cold air to dry.

HPLC activity profiling

The method described by Adams et al. [18] was used for the profiling of *S. repens* extract. Briefly, a 10 mg/ml sample in dimethyl sulfoxide (DMSO) was prepared, filtered through 0.45 µm sterile filters for HPLC fractionation. A Gilson 215 liquid handler with a Gilson 819 injection module and 50 µl loop was used as the autosampler, connected to an HPLC system consisting of a 1100 series low-pressure mixing pump with degasser module, column oven, and a 1100 series PDA detector (Agilent, Waldbronn, Germany). The mobile system used was made up of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). Separation conditions: Sunfire RP-18 column (3.5 µm, 3 mm × 150 mm i.d.; Waters GmbH, Eschborn, Germany), water (A) 90-0% in acetonitrile (B) over 30 min, 100% acetonitrile (B) for 5 min.

The flow rate was 0.5 ml/min, and the injection volume was 35 µl (350 µg extract in DMSO). Thirty-five one-minute fractions were collected in 96 deep well plates during the run (Screenmates 96 well, Matrix Technology, Hudson, USA) and dried in a GeneVac EZ-2 Plus evaporator (Genevac Ltd., Ipswich, UK). The samples were redissolved in 5 µl of DMSO and diluted with 95 µl phosphate buffer solution (PBS) buffer (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) for microfraction antileishmanial screening.

***In vitro* antileishmanial activity evaluation of the microfractions and isolated compound**

The anti-leishmanial activity of the microfractions and isolated compound was evaluated by the method described by Adams et al. [18] at two different concentrations (5 µg/ml) and (0.8 µg/ml) against *L. donovani* amastigotes (MHOM/ET/67/L82) in a 96-well microtiter plate. The plate was read in a spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Synnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm. Flouresence development was measured and expressed as a percentage of the control. The IC_{50} value was determined from the sigmoidal inhibition curve and miltefosine was used as a positive control.

Cytotoxicity evaluations of 12-methoxycarnosic acid against L6-cells

Assays were performed in 96-well microtiter plates, as previously discussed by Adams et al. [18]. The plates were incubated for 72 hours and thereafter inspected under an inverted microscope to assure growth of the controls and sterile conditions. A volume of 10 µl of resazurin was then added to each well and the plates incubated for a further 2 hours. The plates

were then evaluated as described above. The IC_{50} value was calculated from the sigmoidal inhibition curve using SoftmaxPro software and podophylotoxin was used as a positive control.

Isolation of the active compound

The semi-preparative HPLC purification of the DCM/MeOH (1:1) crude extract of *Salvia repens* whole plant was performed with an HPLC 1200 series consisting of a low-pressure mixing pump with degasser module, a column oven and a PDA detector (Agilent, Waldbronn, Germany). A 100 mg/ml sample in DMSO was prepared and filtered through a 0.45 µm Millipore (Bedford, MA, USA) membrane filter and several repititions of 400 µl injections of the sample were made. The separation was performed on a Waters Sunfire RP-18 column (10 × 150 mm i.d.; 10 µm; Waters GmbH, Ireland). The mobile system used was similar to that used for the HPLC activity profiling of the extract and made up of water (A) and acetonitrile (B). The elution gradient was linear from A:B (90:10) to (0:100) in 30 min at a flow rate of 6.0 ml/min followed by washing and returning to the initial elution conditions (90:10) over 5 min. The separation of the active component was detected at 280 nm. The targeted fraction containing the compound of interest was collected and dried in a Genevac evaporator overnight to afford compound **P3-1**. The structure of this compound was determined by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopyand mass spectrometry (MS) analysis as well as by comparison with the published data and was identified as 12-methoxycarnosic acid (**P3-1**) [19]. Using 800 mg of crude extract 10 mg of pure compound **P3-1** was isolated. The purity was >97% determined by HPLC.

Results and discussion

In our effort to identify potential antileishmanial compound/s from the DCM/MeOH (1:1) extract of *Salvia repens* whole plant, an analytical HPLC-based activity profiling technique [18] was used to fractionate the *S. repens* extract in a 96 deep-well microtiter plate. The antileishmanial activity was in fraction 27 (figure **3.1**). The HPLC chromatogram at 280 nm depicted in figure **3.1** made it possible to identify the active peak responsible for the observed antileishmanial activity. Using semipreparative HPLC, 12-methoxycarnosic acid (**P3-1**), a known compound, was isolated and identified from its ^{1}H and ^{13}C NMR spectra (Appendix 2, Page 125 - 126) and by comparison of the NMR and MS data with that published in the literature [19] and its structure is indicated in figure **3.2**. The compound has been isolated previously from *S. microphyllla* [20] and *Rosmarinus officinalis* [19, 21].

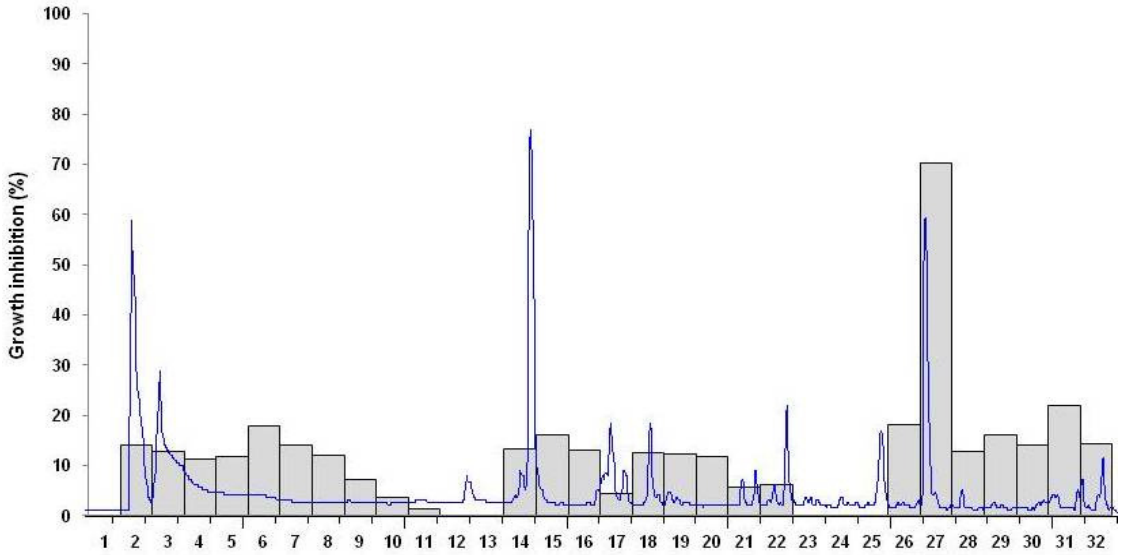
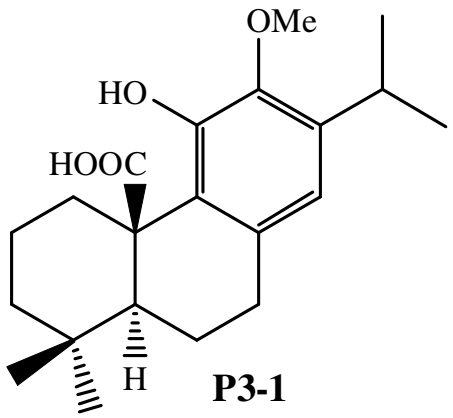


Figure 3.3 Antileishmanial activity trace of a DCM/MeOH (1:1) extract of the whole plant of *Salvia repens* fractions and UV trace (280 nm) of the one minute fractions tested against axenically grown *L. donovani* amastigotes at lower concentration (0.8 µg/ml).

The antileishmanial activity of 12-methoxycarnosic acid (**P3-1**) was evaluated against axenic *Leishmania donovani* amastigotes and showed an IC_{50} value of 0.75 µM. Miltefosine (the positive control) had an IC_{50} of 0.19 µM. The selectivity index (SI), calculated as the ratio between IC_{50} value for cytotoxicity and IC_{50} value for *L. donovani* amastigotes for compound (**P3-1**) was 23.2.



P3-1

Figure 3.4 The chemical structure of 12-methoxycarnosic acid (**P3-1**) isolated from the DCM/MeOH (1:1) extract of the whole plant of *Salvia repens*

In recent years, the Lamiaceae plants of the genus *Salvia* have been extensively investigated for their biological and pharmacological properties. Phytochemical studies of several *Salvia* species have led to the isolation of various phytochemical constituents mainly abietane diterpenoids such as carnosol, carnosic acid, rosemarinic acid [22] which have shown antioxidant [23] and cytotoxic [24] activities. The antileishmanial [4] and antiplasmodial [25] activities of abietane diterpenoids have also been reported.

Carnosic acid have shown to posses good peroxyl and hydroperoxyl radical scavenging activity, since it inhibits the formation of hydroxyl radicals and chelate metals and also scavenge H_2O_2 [23]. In the other study, carnosic acid has been shown to inhibit lipid absorption [26]. Previous reports have shown that **P3-1** has very interesting biological activities. Murata and co-authors [27] reported that **P3-1** resulted in a 66.7% binding inhibition of 5α-dihydrotestosterone (DHT) to the androgen receptor (AR) at 5 μM and the inhibition of 5α-reductase (IC_{50}, 61.7 μM), thereby playing a significant role in promoting hair growth. To the best of our knowledge this is the first report on the antileishmanial activity of 12-methoxycarnosic acid (**P3-1**).

Conclusion

The use of HPLC-based activity profiling made it possible to identify the active compound responsible for antileishmanial activity. Compound (**P3-1**), 12-methoxycarnosic acid isolated from *Salvia repens* showed in *vitro* antileishmanial activity against axenic *L. donovani* amastigotes. In addition, compound **P3-1** has good selectivity. Modifications to the structure of this compound could result in the development of new analogues with much more improved activity against this parasite.

Acknowledgements

The authors would like to thank the Council for Scientific and Industrial research (CSIR), and the Swiss Confederation for financial support under the Swiss South African Joint Research Programme (grant JRP 03), and the South African National Biodiversity Institute (SANBI) for the identification of plant species.

Conflict of interest

The authors declare that they have no conflict of interest.

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CHAPTER 4

ANTIPROTOZOAL SCREENING OF 60 SOUTH AFRICAN PLANTS, AND THE IDENTIFICATION OF THE ANTITRYPANOSOMAL GERMACRANOLIDES SCHKUHRIN I AND II

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Abstract

Two hundred and seven extracts were prepared from sixty plants from South Africa and screened for *in vitro* activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. For the 21 extracts which inhibited the growth of one or more parasites more than 95% at 10 µg/mL, the IC_{50} values against all four protozoal parasites and cytotoxic IC_{50} values against L6 myoblast were determined. Amongst the most notable results were the activities of *Psoralea pinnata* (IC_{50} of 0.15 µg/mL), *Schkuhria pinnata* (2.04 µg/mL) and *Vernonia mespilifolia* (1.01 µg/mL) against *Trypansoma brucei rhodesiense.* HPLC based activity profiling was used to identify the active constituents in the extracts, and the germacranolides sesquiterpene lactones schkuhrin **I** and **II** from *Schkuhria pinnata*, and cynaropicrin from *Vernonia mespilifolia* were identified, with IC_{50} values of 0.9, 1.5, and 0.23 µM, respectively.

Keywords: Screening, plant extract library, antiprotozoal activity, sesquiterpene lactones, Asteraceae, *Psoralea pinnata*, *Schkuhria pinnata*, *Vernonia mespilifolia*, *Trypansoma brucei rhodesiense, Trypanosoma cruzi, Leishmania donovani, Plasmodium falciparum,* plant extracts, parasites.

Introduction

Neglected tropical diseases (NTDs) caused by *Leishmania* species (leishmaniasis), *Trypanosoma brucei rhodesiense* (sleeping sickness) and *Trypanosoma cruzi* (Chagas disease) are life threatening and represent a risk to large parts of the populations in poor tropical countries. These diseases affect almost 21.3 million people worldwide, mostly in economically challenged regions [1]. It is estimated that these three diseases are responsible for over 110,000 deaths every year [2]. In addition, malaria caused by *Plasmodium* species

was responsible for approximately 216 million infections and over 655,000 deaths in 2010 [3]. The control of these parasitic infections relies on a few chemotherapeutic agents, most of which were discovered many decades ago [4] and pose many challenges due to adverse side effects, long treatment cycles, poor efficacy, high costs, the occurrence of drug resistances, and limited availability [1]. Therefore, the discovery of novel, safe and effective antiprotozoal agents is an urgent need.

Natural products play a significant role in the discovery of new drug leads because of the unmatched availability of chemical diversity. We previously reported a screen of 300 extracts from plants traditionally used to treat malaria and their antiprotozoal effects against *L. donovani*, *T. b. rhodesiense*, *P. falciparum* and *T. cruzi in vitro* [5]. This work is a continuation of the project, where 207 further extracts have been tested. Furthermore, we report the identification of three antitrypanosomal sesquiterpene lactones from the most active extracts, which were identified by HPLC based activity profiling [6].

Materials and methods

General experimental procedures

Plant collection and identification

Sixty plant species were collected between 1996 and 2002 at a variety of locations in South Africa. The selected plant species were based on their traditional uses against parasitic diseases (unpublished work). The species were identified by personnel of the South African National Biodiversity Institute (SANBI) where the voucher specimens are deposited.

Plant extract preparations

The plant parts (roots, leaves, twigs, fruit, and stem bark) were dried in an oven at 30-60 ºC and extracts from finely ground plant parts were prepared as previously described [5]. Stock solutions were prepared at 10 mg/mL in DMSO and stored at -80 °C until use. Dilutions for biological testing were freshly prepared in culture medium on the day of testing (< 1% DMSO).

Isolation and purification of active compounds P4-1, P4-2, and P4-3

For compounds **P4-1**, **P4-2**, and **P4-3**, semi-preparative HPLC purification was performed using an Agilent HPLC 1200 series consisting of a low-pressure mixing pump with degasser module, column oven, PDA detector (Agilent, Waldbronn, Germany). The extract of the whole plant of *Schkuhria pinnata* (Lam.) Cabrera and *Vernonia mespilifolia* Less. (100 mg/mL in DMSO, respectively) were prepared by dissolving 1.0 g of DCM/MeOH (1:1) extract, filtered through 0.45 µm Millipore (Bedford, MA, USA) membrane filters and several repetitions of 200 µL injections made. The separation was performed on a Waters Sunfire RP-18 column (10 × 150 mm i.d.; 10 µm; Waters GmbH, Ireland). The mobile system used was made up of water (A) and acetonitrile (B). The elution gradient was linear from A:B (90:10) to (0:100) in 30 min at a flow rate of 6.0 mL/min, followed by 5 min acetonitrile washing and returned to the initial elution conditions (90:10) over 5 min. The separation of all components was detected at a wavelength of 254 nm.

Biological assays

All extracts were screened as previously described [5] against *P. falciparum* (NF-54 strain), *T. b. rhodesiense* (STIB 900 strain), *T. cruzi* trypomastigote forms (Tulahuen strain), and *L. donovani* (strain MHOM/ET/67/L82) at 10 (high concentration) and 2 µg/mL (low

concentration). Half maximal inhibition concentrations (IC_{50}) were determined by serial dilution, and represent the mean of at least two independent experiments. HPLC-based activity profiling was done as previously described [7]. Positive controls, melarsoprol (*T. b. rhodesiense*), benznidazole (*T. cruzi*), miltefosine (*L. donovani*), chloroquine (*P. falciparum*), and podophyllotoxin (L6 cells) were used. The purity of all controls was > 95% according to the suppliers.

In vitro test against *Trypanosoma brucei rhodesiense*

Trypanosoma brucei rhodesiense (STIB 900) were grown in axenic medium as previously described [8]. The compounds were tested using a modified Alamar Blue assay protocol [9] to determine the 50% inhibitory concentration (IC_{50}). Serial three-fold drug dilutions were prepared in 96-well microtiter plates and 50 μL of *T. b. rhodesiense* STIB 900 bloodstream forms were added to each well except for the negative controls. Melarsoprol (Arsobal®, Sanofi-Aventis, Meyrin, Switzerland) was used as a reference drug. After 70 h of incubation Alamar blue marker (12.5 mg resazurin dissolved in 100 mL distilled water) was added. The plates were then incubated for an additional 2 to 5 h. A Spectramax Gemini XS micro plate fluorescence reader (Molecular Devices Cooperation, Sunnyvale, CA) with an excitation wavelength of 536 nm and an emission wavelength of 588 nm was used to read the plates. The IC_{50} values were calculated from the sigmoidal growth inhibition curves using Softmax Pro software (Molecular Devices).

In vitro testing against *Plasmodium falciparum*

A modification of the [^{3}H]-hypoxanthine incorporation assay was used to determine the intra-erythrocytic antiplasmodial activity [10] of the extract library and purified compounds in 96 well plates. Chloroquine (Sigma-Aldrich) and artesunate (Mepha, Switzerland) were used as

standard drugs. Briefly, infected human red blood cells in RPMI 1640 medium (100 µL per well with 2.5% haematocrit and 0.3% parasitaemia) were exposed to two-fold serial drug dilutions in 96-well microtiter plates. After 48 h incubation, 0.5 µCi [^{3}H]-hypoxanthine was added to each well. The plates were incubated for further 24 h before being harvested using a Betaplate cell harvester (Wallac, Zürich, Switzerland). The radioactivity was counted with a Betaplate liquid scintillation counter (Wallac) as counts per minute per well at each drug concentration and compared to the untreated controls. IC_{50} values were calculated from sigmoidal inhibition curves using Microsoft Excel. All assays were run in duplicate and repeated three times [10].

***In vitro* cytotoxicity testing**

Cytotoxicity was assessed using a similar Alamar Blue assay protocol [8] whereby 4000 rat myoblast cells/well were seeded in RPMI 1640 medium. All following steps were according to the *T.b.rhodesiense* protocol. Podophyllotoxin (Sigma-Aldrich) was used as the reference drug.

Results and Discussion

A total of 207 plants extracts from 67 plant species were assessed for antiprotozoal activity against four protozoan parasites at 10 and 2 µg/mL, respectively (Appendix 1**,** Table **4.1**, 111 - 122). The most active extracts (≥ 95% parasitic growth inhibition) from the preliminary screening were subjected to the IC_{50} value determination study against the parasites which were most susceptible to. Extracts which exhibited an IC_{50} value ≤ 5.0 µg/ml were considered active and were subjected to HPLC-based activity profiling technique to identify potential antiprotozoal compounds. The lipophillic extracts of *Psoralea pinnata* and *Drypetes gerrardii* exhibited very good antiprotozoal activity against *T. b. rhodesiense* (IC_{50}, 0.15 ±

0.02 and 0.31 ± 0.02 µg/mL) and *P. falciparum* (IC_{50}, 0.50 ± 0.23 µg/mL), respectively (Appendix 1, Table **4.2**, Page 123 - 124). In addition, these extracts showed the best selectivity index values ranging between 97 and 139 indicating their selectivity towards killing the parasites with very little toxicity towards the myoblasts L-6 cells. *Vernonia mespilifolia* Less., *Oedera genistifolia*, *Abrus precatorius africanus* and *Ekebergia capensis* also exhibited IC_{50} values ranging between 1.0 and 1.7 µg/mL against *T. b. rhodesiense* and *L. donovani*, however, with moderate selectivity indices ranging from 24 to 48.

The active extracts of *Psoralea pinnata*, *Schkuhria pinnata* and *Vernonia mespilifolia* were further studied to identify their active constituents. For the purpose of this study we report the identification of active compounds from *S. pinnata* and *V. mespilifolia* by the aid of HPLC based activity profiling technique. The one minute microfractions from the active DCM/MeOH extract from *Schkuhria pinnata* were tested against *T. b. rhodesiense,* and it was shown that the activity was concentrated in the active time windows of minutes 14 and 16 (**Figure 4.1**), which showed 100 % inhibition. These wells both contained one main substance, which were isolated by semi-preparative HPLC and structurally elucidated by NMR, high resolution MS, and comparison of spectral data to literature [11]. Fraction 14 was shown to contain schkuhrin I (**P4-1**) and fraction 16, schkuhrin II (**P4-2**). The two structurally similar germacranolides showed antiprotozoal activity against *T. b. rhodesiense* with IC_{50} values of 0.86 and 1.50 µM, respectively and antiplasmodial activity with IC_{50} values of 2.05 and 1.67 µM, respectively (**Table 4.3**).

Table 4.3 Antiprotozoal activity (IC_{50} values in µM) of schkuhrin I (**P4-1**), schkuhrin II (**P4-2**) and cynaropicrin (**P4-3**) against *T. b. rhodesiense*, *T. cruzi*, *L. donovani* and *P. falciparum*

	T. b. rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum*		*Cytotox. L6*
	IC_{50}	**SI**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**
Schkuhrin I (**P4-1**)	0.86	6.12	16.4	0.32	39	0.13	2.05	2.57	5.26
Schkuhrin II (**P4-2**)	1.50	6.02	26.96	0.33	65	0.14	1.67	5.41	9.03
Cynaropicrin (**P4-3**)	0.23	5.61	5.14	0.25	1.56	0.83	1.56	0.83	1.29
Melarsoprol	0.01								
Benznidazole			2.25						
Miltefosine					0.47				
Chloroquine							0.015		
Podophyllotoxin									0.017

The HPLC activity profile of the DCM/MeOH extract of *V. mespilifolia* showed significant antiprotozoal activity in fraction 12 and 13 against *T. b. rhodesiense* (**Figure 4.2**). The major compound in these fractions was isolated and by use of NMR, high resolution MS and comparison to literature data [12] identified as the known sesquiterpene lactone cynaropicrin (**P4-3**). The IC_{50} values of **P4-3** were 0.23 µM against *T. b. rhodesiense* and 1.56 µM against *P. falciparum*. *L. donovani* exhibited an IC_{50} value of 1.56 µM whereas *T. cruzi* was the least susceptible parasite with an IC_{50} value of 5.14 µM.

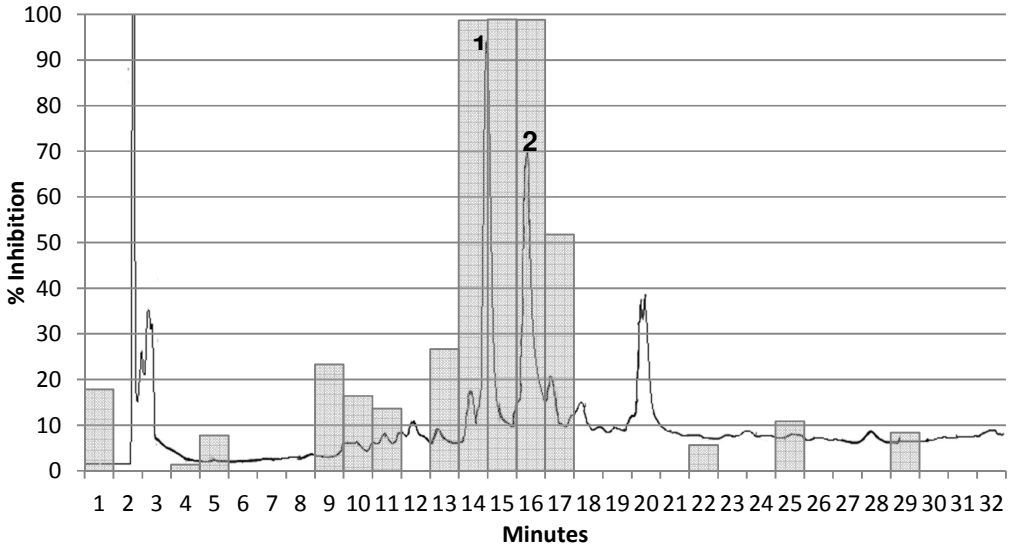

Figure 4.1 HPLC-based activity profiling of *Schkuhria pinnata* organic extract against *T. b. rhodesiense*. The bar graphs show the inhibitory activity of individual HPLC fractions collected from a single separation of the crude extract. The HPLC chromatogram was measured at 254 nm

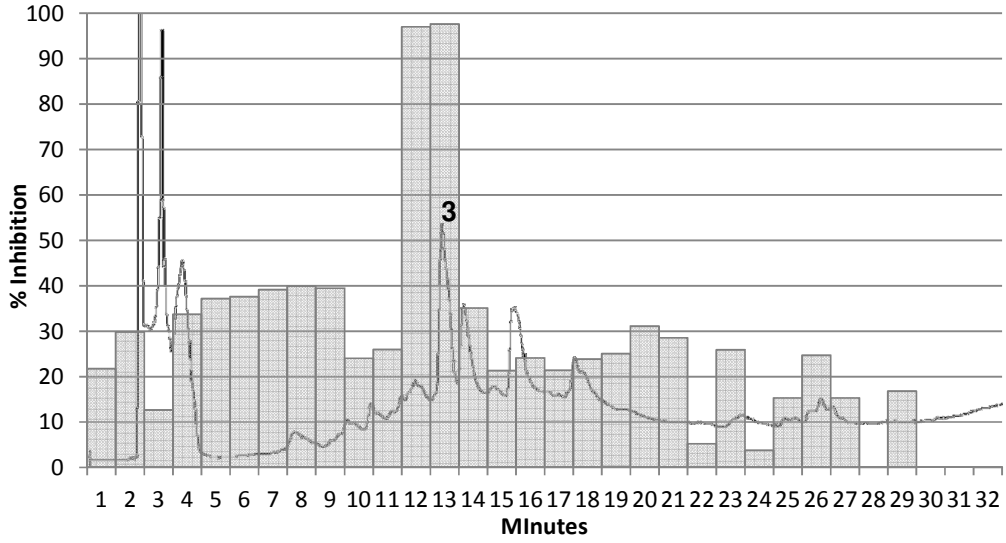

Figure 4.2 HPLC-based activity profiling of *Vernonia mespilifolia* organic extract against *T. b. rhodesiense*. The bar graphs show the inhibitory activity of individual HPLC fractions collected from a single separation of the crude extract. The HPLC chromatogram was measured at 254 nm.

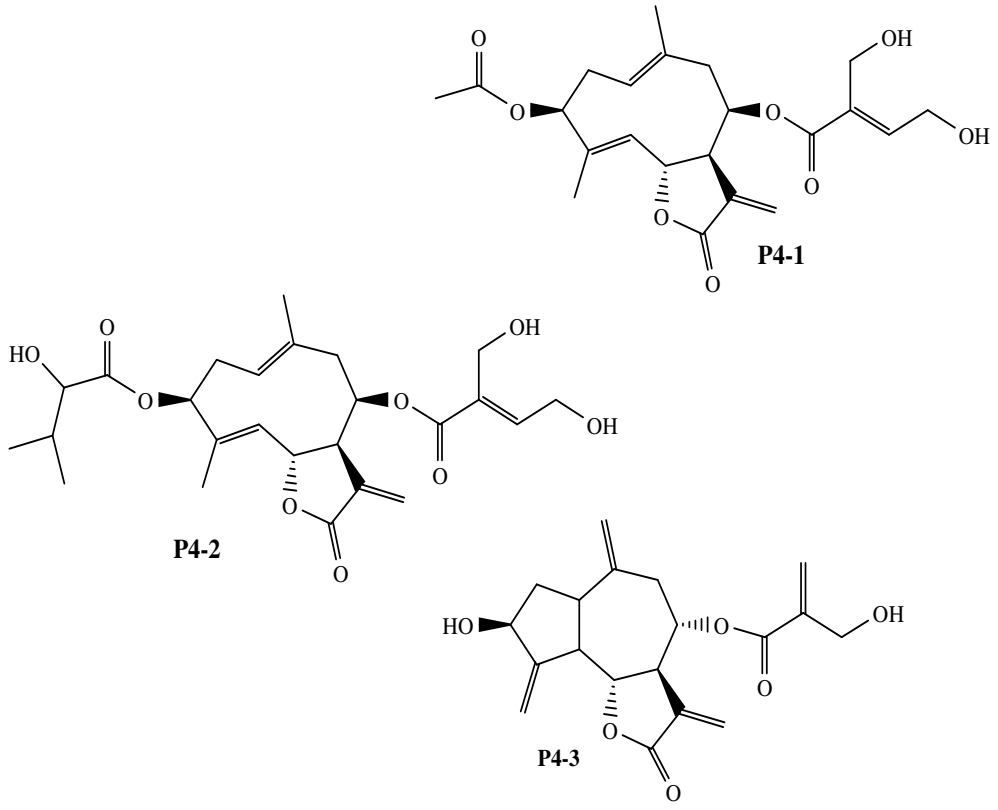

Figure 4.3 Structures of the isolated compounds, schkuhrin I (**P4-1**) and schkuhrin II (**P4-2**) from *Schkuhria pinnata* and cynaropicrin (**P4-3**) from *Vernonia mespilifolia* crude extracts

This study is a continuation of our efforts of collecting and screening South African plants against protozoan parasites [5]. The results of a large screen of 207 extracts are shown (Appendix 1, Table **4.1**, Page 112 - 123). Furthermore, by using HPLC based activity profiling the sesquiterpene lactones schkuhrin I (**P4-1**) and II (**P4-2**) from *Schkuhria pinnata*, and cynaropicrin (**P4-3**) from *Vernonia mespilifolia*, were identified as potent antiprotozoal agents. The powdered leaf of *S. pinnata* has been used as a remedy for malaria, influenza and

colds [13] as well as insecticides, and particularly to kill fleas [14]. Both schkuhrin I (**P4-1**) and II (**P4-2**) have been isolated from the same species and other *Schkuhria* species but their antiprotozoal activities especially against these parasites have not been reported previously. Some of the species belonging to the genus *Vernonia* have been used in folk medicine to treat malaria [15]. *V. mespilifolia* Less. is used traditionally by the Zulu population in South Africa to treat malaria related feverish conditions [16].

Sesquiterpenes are the largest known group of natural products [17] and are a chemical marker for the largest plant family, the Asteraceae [18]. Numerous sesquiterpene lactones have been tested for antiprotozoal activity *in vitro*. Schmidt et al. [19] showed in an antitrypanosomal-QSAR study of 40 STLs, and in 2012 supplied two excellent reviews of antiprotozoal *in vitro* effects of 883 plant derived natural products including 83 sesquiterpene lactones [17, 20]. This study is the first report of the antiprotozoal effects of **P4-1** and **P4-2**, whereas the antitrypansomal effects of **P4-3** had already been described by Zimmermann et al. [7]. In fact, **P4-3** was the first and so far the only plant derived compound to inhibit *T. b. rhodesienese in vivo*. The mode of action of **P4-3** was shown to be its ability to form stable bis-adducts with trypanothione via a Michael addition, thus depleting the parasites of their only means of redox control [21].

With this screening study we report recent *in vitro* results of a plant extract screen. This may provide a basis for selecting plants in the future for further phytochemical and bioactivity studies. Furthermore we report the *in vitro* antiprotozoal effects of three compounds, which will contribute to a better understanding of sesquiterpene lactones antiprotozoal structure-activity relationships.

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

The authors would like to thank the Council for Scientific and Industrial research (CSIR), the Department of Science and Technology and the Swiss Confederation for financial support under the Swiss South African Joint Research Programme (grant JRP 03) and the South African National Biodiversity Institute (SANBI) for the identification of plant species.

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CHAPTER 5

GENERAL CONCLUSIONS

In this study, over 507 extracts from 174 South African plant species used for the treatment of parasitic diseases, mainly malaria and associated symptoms such as fever were selected and screened in a medium throughput screen to identify and select active plant species for the isolation of active compounds with the potential to act against *T. b. rhodesiense*, *T.cruzi*, *L. donovani* and *P. falciparum*. Of the plant species used in the study, 107 showed very promising antiprotozoal activities by exhibiting more than 95% growth inhibition against *T. b. rhodesiense*, *T.cruzi*, *L. donovani* and *P. falciparum* when tested at 10 µg/ml. The antiprotozoal activities were quantified by subjecting the extracts of these plant species to IC_{50} and cytotoxicity determination assays, thereby determining not only their activity, but their selectivity as well, taking into account the toxicity of the compounds used in the bioassays. Plants extracts with a very low selectivity index may not necessarily be good candidates for further isolation simply because the observed activity might be due to a general toxicity of the compounds contained in the extracts. On the other hand, the active compound may not necessarily be the compound responsible for the toxicity of the extract. Nevertheless, plants with a high selectivity index are more suited to phytochemical studies. A library of South African plant species with potential antiprotozoal activity was thus identified from this work and would be available for those chemists wanting to undertake phytochemical studies in the search for lead compounds with antiprotozoal activity. As a follow up study, *Salvia repens, Schkuhria pinnata* and *Vernonia mespilifolia* with selectivity indices of were chosen to be studied phytochemically and to isolate the active components in them.

With the aid of the HPLC-based activity profiling technique, four active compounds (**P3-1**, **P4-1**, **P4-2** and **P4-3**) were isolated from the extracts of *Salvia repens*, *Schkuhria pinnata* and *Vernonia mespilifolia*. The isolated compounds and in particular, the sesquiterpene lactones showed very promising antiprotozoal activity against *Trypanosoma brucei rhodesiense*. Of the four, cynaropicrin (**P4-3**) from the leaves of *V. mespilifolia* was the most active. Toxicity studies against the rat skeletal myoblast cells indicated that these compounds are quite cytotoxic. Further work, such as structural modifications to these sesquiterpene lactones is important to determine whether the activity has to do with certain functional groups on the molecule and whether or not the molecule retains it acitivty, should the molecule be made less cytotoxic by the derivatisation of the functional groups.

Further phytochemical work on other lead plants from this study can also be carried out either using conventional Natural Products Chemistry approaches or the HPLC-based activity profiling as described in this thesis.

Appendix 1

Table 4.1 Medium Throughput screening (MTS) results of 300 South African plants extracts against *Trypanosoma brucei rhodesience*, *Trypanosoma cruzi*, *L. donovani* and *Plasmodium falciprum.* Antiprotozoal activity reported in percentage (%) growth inhibition.

					T. b. rhodesience		***T. cruzi***		***L. donovani***		***P. falciparum***	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Acanthaceae	*Asystasia gangetica T.Anderson*	P05622b	Twigs	DCM/MeOH (1:1)	5.6	0.0	0.0	0.0	27.5	0.0	39.5	3.3
Fabaceae	*Acacia nilotica (L.) kraussianna*	P12859b	Twigs	DCM/MeOH (1:1)	18.0	17.4	6.3	4.7	22.2	11.1	43.0	0.0
Fabaceae	*Acacia nilotica (L.) kraussianna*	P12859c	Twigs	Aqueous	6.2	9.4	9.9	1.3	18.2	2.2	0.0	0.0
Fabaceae	*Acacia tortilis (Forssk) Hayne*	P12869b	Whole plant	DCM/MeOH (1:1)	16.7	4.3	12.4	3.3	27.9	6.7	58.9	4.3
Amaranthaceae	*Achyranthes aspera L.*	P15190b	Whole plant	DCM/MeOH(1:1)	11.9	9.7	0.0	0.0	35.8	0.0	52.5	0.0
Rutaceae	*Agathosma apiculata G.Mey.*	P09995b	Whole plant	DCM/MeOH (1:1)	99.1	0.0	0.0	0.4	72.9	14.2	80.1	21.4
Rutaceae	*Agathosma puberula (Steud.) Forc.*	P02011a	Roots	DCM	98.4	7.4	0.0	0.0	67.5	8.5	57.6	1.0
Rutaceae	*Agathosma puberula (Steud.) Forc.*	P02011b	Roots	DCM/MeOH (1:1)	0.0	1.3	0.0	0.0	29.4	0.8	33.8	0.7
Rutaceae	*Agathosma puberula (Steud.) Forc.*	P02022a	Stem bark	DCM	3.0	8.0	10.3	2.2	52.5	5.3	94.6	9.3
Rutaceae	*Agathosma puberula (Steud.) Forc.*	P02022b	Stem bark	DCM/MeOH (1:1)	4.6	0.0	3.9	0.0	17.3	4.2	25.0	14.4

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Asphodelaceae	*Ageratum conyzoides L.*	P12944b	Whole plant	DCM/MeOH(1:1)	3.6	0.9	9.4	3.4	27.0	16.6	36.2	0.0
Asphodelaceae	*Ageratum conyzoides L.*	P12944c	whole plant	Aqueous	0.0	0.0	0.0	8.1	39.9	20.4	4.3	4.0
Apiaceae	*Alepidea amatymbica Eckl. & Zeyh.*	P02873b	Whole plant	DCM/MeOH (1:1)	1.7	0.0	0.0	0.0	89.2	23.3	56.4	1.2
Asphodelaceae	*Aloe ferox Mill.*	P01713a	Fruits	DCM	10.4	0.0	1.7	0.0	7.2	0.8	14.0	0.0
Asphodelaceae	*Aloe ferox Mill.*	P01713b	Fruits	DCM/MeOH (1:1)	14.7	0.0	0.0	5.0	17.1	8.7	1.2	0.0
Asphodelaceae	*Aloe ferox Mill.*	P01713c	Fruits	Aqueous	0.6	6.9	6.9	4.9	13.3	0.0	0.0	0.0
Asphodelaceae	*Aloe ferox Mill.*	P03153b	Whole plant	DCM/MeOH (1:1)	8.6	4.0	0.0	0.0	41.6	11.4	59.0	5.0
Asphodelaceae	*Aloe marlothii A.Berger*	P00054b	Leaves	DCM/MeOH (1:1)	13.4	9.6	0.0	0.0	5.2	0.0	0.0	3.9
Asphodelaceae	*Aloe marlothii A.Berger*	P00054d	Leaves	Aqueous	15.4	8.5	0.0	0.0	21.5	9.2	3.1	0.0
Asphodelaceae	*Aloe marlothii A.Berger*	P00770b	Whole plant	DCM/MeOH (1:1)	0.0	0.0	0.0	0.0	21.0	2.4	15.8	0.6
Annonaceae	*Annona senegalensis*	P01034b	Leaves	DCM/MeOH (1:1)	0.0	2.6	0.3	0.0	11.2	0.0	92.5	0.0
Gentianaceae	*Anthocleista grandiflora Gilg*	P01455c	Leaves	Aqueous	0.0	2.5	0.0	0.0	16.6	3.3	2.2	0.0
Gentianaceae	*Anthocleista grandiflora Gilg*	P01455a	Leaves	DCM	43.3	23.6	0.0	0.0	31.6	5.1	26.4	0.0
Gentianaceae	*Anthocleista grandiflora Gilg*	P01455b	Leaves	DCM/MeOH (1:1)	18.0	20.6	0.0	0.0	20.4	16.0	2.4	0.0
Annonaceae	*Artabotrys brachypetalus Benth.*	P02239b	Twigs/Leaves	DCM/MeOH (1:1)	3.5	6.1	0.0	0.0	16.3	11.5	17.3	2.7

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Annonaceae	*Artabotrys brachypetalus Benth.*	P02239c	Twigs/Leaves	Aqueous	2.0	0.8	0.0	1.3	15.8	3.1	0.0	0.0
Annonaceae	*Artabotrys monteiroae Oliv.*	P18314b	Leaves	DCM/MeOH (1:1)	99.4	0.0	9.2	7.9	25.0	5.2	40.5	0.0
Annonaceae	*Artabotrys monteiroae Oliv.*	P18314c	Leaves	Aqueous	0.0	0.3	15.0	5.3	17.6	10.3	0.0	0.0
Asteraceae	*Artemisia afra Jacq. ex Willd.*	P00484a	Leaves	DCM	98.2	0.0	0.0	0.0	100.0	25.9	89.5	8.0
Asteraceae	*Artemisia afra Jacq. ex Willd.*	P00484b	Leaves	DCM/MeOH (1:1)	96.7	0.2	0.0	0.0	100.0	43.5	77.1	3.0
Asteraceae	*Artemisia afra Jacq. ex Willd.*	P00484c	Leaves	MeOH	96.2	5.3	0.0	0.0	81.2	0.5	35.3	0.0
Asparagaceae	*Asparagus virgatus Baker*	P08216b	Whole plant	DCM/MeOH(1:1)	10.5	9.2	0.0	10.9	34.6	14.1	54.9	3.2
Acanthaceae	*Asystasia gangetica T.Anderson*	P05623b	Leaves	DCM/MeOH (1:1)	0.0	0.0	1.7	0.0	18.5	0.0	99.8	51.4
Lecythidaceae	*Barringtonia racemosa (L.) Roxb.*	P15194c	Leaves	Aqueous	0.0	5.5	3.0	0.0	14.6	8.7	0.0	0.8
Lecythidaceae	*Barringtonia racemosa (L.) Roxb.*	P15194b	Leaves	DCM/MeOH (1:1)	3.1	0.0	4.8	3.8	24.9	2.7	33.1	6.0
Lecythidaceae	*Barringtonia racemosa (L.) Roxb.*	P15193b	Twigs	DCM/MeOH (1:1)	31.6	5.3	6.5	2.0	26.1	0.0	26.6	0.0
Apiaceae	*Berula erecta (Huds.) Coville*	P05646b	Whole plant	DCM/MeOH(1:1)	17.2	9.1	0.0	0.0	35.8	5.0	44.3	6.2
Asteraceae	*Bidens pilosa L.*	P00071b	Leaves	DCM/MeOH (1:1)	20.3	21.3	0.0	0.0	25.9	14.4	42.9	15.2
Asteraceae	*Bidens pilosa L.*	P00071c	Leaves	MeOH	21.2	17.1	0.0	0.0	37.2	13.8	47.3	4.9
Asteraceae	*Bidens pilosa L.*	P00071d	Leaves	Aqueous	18.9	14.7	0.0	0.0	22.6	14.1	12.8	0.3
Rhizophoraceae	*Bruguiera gymnorhiza (L.) Lam.*	P18322b	Twigs	DCM/MeOH(1:1)	0.0	0.0	0.0	1.1	26.0	9.0	34.9	0.4

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Capparaceae	*Capparis tomentosa Lam.*	P00665a	Leaves	DCM	29.8	23.8	0.0	0.0	39.4	6.7	4.9	0.0
Capparaceae	*Capparis tomentosa Lam.*	P00667a	Stem bark	DCM	7.5	6.3	0.0	0.0	29.6	0.0	14.5	0.0
Capparaceae	*Capparis tomentosa Lam.*	P00669a	Roots	DCM	5.2	5.7	0.0	0.0	17.7	9.4	0.0	3.9
Capparaceae	*Capparis tomentosa Lam.*	P00665b	Leaves	DCM/MeOH (1:1)	5.7	0.0	0.0	0.0	18.8	0.0	21.4	0.0
Capparaceae	*Capparis tomentosa Lam.*	P00667b	Stem bark	DCM/MeOH (1:1)	3.8	17.1	0.0	0.0	10.0	0.0	0.6	0.0
Capparaceae	*Capparis tomentosa Lam.*	P00669b	Roots	DCM/MeOH (1:1)	22.2	3.0	0.0	0.0	32.3	3.8	0.0	0.0
Apocynaceae	*Carissa edulis Vahl*	P00334a	Stem bark	DCM	0.0	6.1	0.0	0.0	36.1	12.1	27.7	3.0
Apocynaceae	*Carissa edulis Vahl*	P00334b	Stem bark	DCM/MeOH (1:1)	8.7	7.0	0.0	0.0	32.9	7.5	14.2	0.0
Apocynaceae	*Carissa edulis Vahl*	P00334c	Stem bark	MeOH	13.5	16.1	0.0	0.0	19.2	0.0	0.0	0.0
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00465a	Seed	DCM	0.0	0.0	0.0	0.0	33.3	10.4	36.5	0.0
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00465b	Seed	DCM/MeOH (1:1)	0.0	11.5	0.0	0.0	19.1	0.0	10.6	0.0
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00465c	Roots	MeOH	0.0	9.4	0.0	0.0	16.4	7.0	8.6	0.0
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00469a	Roots	DCM	57.1	9.7	0.0	0.0	100.0	23.2	99.4	8.8
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00469b	Roots	DCM/MeOH (1:1)	0.0	8.3	0.0	0.0	30.9	0.0	23.5	0.0
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00469c	Leaves	MeOH	2.8	3.1	0.0	0.0	9.2	3.5	17.9	0.0
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00470a	Leaves	DCM	5.2	0.0	0.0	0.0	20.8	0.0	29.5	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00470b	Leaves	DCM/MeOH (1:1)	0.0	7.9	0.0	0.0	13.5	0.0	42.1	8.2
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00465d	Seed	Aqueous	0.0	7.4	2.8	2.1	5.9	4.5	0.0	0.0
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00469d	Roots	Aqueous	0.0	0.0	9.8	0.0	23.7	12.3	0.0	0.0
Celastraceae	*Catha edulis (Vahl) Forssk. ex Endl.*	P00470d	Leaves	Aqueous	0.0	4.4	1.7	6.4	24.3	9.1	92.2	0.0
Apiaceae	*Centella asiatica (L.) Urb.*	P05632b	Leaves	DCM/MeOH (1:1)	0.0	0.0	0.0	2.6	42.9	1.6	38.6	6.8
Rutaceae	*Clausena anisata*	P09997b	Twigs	DCM/MeOH (1:1)	4.7	0.0	0.0	0.0	20.5	7.6	18.1	0.7
Rutaceae	*Clausena anisata*	P09998b	Leaves	DCM/MeOH (1:1)	4.8	0.0	3.9	0.0	30.9	2.8	24.9	0.0
Euphorbiaceae	*Clutia hirsuta E.Mey. ex Sond.*	P11867b	Whole plant	DCM/MeOH (1:1)	8.0	3.9	0.0	0.9	40.1	11.8	68.9	10.2
Euphorbiaceae	*Clutia hirsuta E.Mey. ex Sond.*	P11867c	Whole plant	Aqueous	25.6	15.2	1.3	0.0	20.3	9.1	0.0	0.0
Combretaceae	*Combretum zeyheri Sond.*	P13042b	Twigs	DCM/MeOH (1:1)	9.5	0.0	47.3	0.0	25.8	4.4	33.4	0.0
Combretaceae	*Combretum zeyheri Sond.*	P13042c	Twigs	Aqueous	7.0	0.0	0.0	0.0	10.8	6.9	19.0	0.0
Asteraceae	*Conyza albida Spreng.*	P12954b	Whole plant	DCM/MeOH (1:1)	99.9	0.0	0.0	2.8	62.8	9.7	97.3	6.2
Asteraceae	*Conyza albida Spreng.*	P12954c	whole plant	Aqueous	0.0	3.5	0.0	1.3	21.2	11.2	1.0	0.0
Asteraceae	*Conyza podocephala DC.*	P03063b	Whole plant	DCM/MeOH (1:1)	6.4	4.5	0.0	0.0	53.2	4.0	98.6	12.4
Asteraceae	*Conyza scabrida DC.*	P03168b	Flowers/Buds	DCM/MeOH (1:1)	1.7	6.0	0.0	0.0	93.0	11.9	59.6	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Asteraceae	*Conyza scabrida DC.*	P03169b	Twigs	DCM/MeOH (1:1)	1.8	15.3	0.0	0.0	45.4	17.8	40.8	0.0
Asteraceae	*Conyza scabrida DC.*	P03170b	Leaves	DCM/MeOH (1:1)	0.0	2.6	0.0	0.0	100.0	4.8	52.6	7.2
Amaryllidaceae	*Crinum macowanii Baker*	P05637b	Flowers/Buds	DCM/MeOH (1:1)	51.6	25.8	1.2	3.4	36.0	5.5	46.8	0.0
Amaryllidaceae	*Crinum macowanii Baker*	P05637c	Flowers/Buds	Aqueous	0.0	0.0	0.0	0.0	2.1	0.0	1.6	0.0
Fabaceae	*Crotalaria burkeana Benth.*	P00417b	Roots	DCM/MeOH (1:1)	25.4	9.2	0.0	0.0	22.3	5.2	20.5	0.0
Fabaceae	*Crotalaria burkeana Benth.*	P00418b	Seed	DCM/MeOH (1:1)	18.0	7.7	0.0	0.0	63.0	10.4	65.6	5.3
Fabaceae	*Crotalaria burkeana Benth.*	P00417c	Leaves	MeOH	6.2	4.6	0.0	0.0	28.9	9.1	4.7	0.0
Fabaceae	*Crotalaria burkeana Benth.*	P00417d	Leaves	Aqueous	11.2	7.1	0.0	0.0	22.6	0.0	0.0	0.0
Fabaceae	*Crotalaria burkeana Benth.*	P00418d	Roots	Aqueous	6.8	3.2	0.0	0.0	21.9	6.7	0.0	0.0
Euphorbiaceae	*Croton gratissimus*	P00010c	Leaves	MeOH	14.1	13.0	0.0	0.0	31.2	4.7	23.9	13.1
Euphorbiaceae	*Croton gratissimus*	P00010d	Leaves	Aqueous	4.4	17.3	0.0	0.0	10.4	3.2	0.0	0.0
Euphorbiaceae	*Croton menyhartii Pax*	P12951b	Leaves	DCM/MeOH (1:1)	98.6	2.1	8.1	0.0	65.6	6.7	100.0	27.4
Euphorbiaceae	*Croton menyhartii Pax*	P12952b	Twigs	DCM/MeOH (1:1)	97.9	10.2	0.1	1.5	55.9	20.1	100.1	32.8
Euphorbiaceae	*Croton menyhartii Pax*	P12951c	Leaves	Aqueous	16.2	7.2	0.0	12.3	28.5	16.8	3.6	0.0
Euphorbiaceae	*Croton menyhartii Pax*	P12952c	Twigs	Aqueous	3.8	4.9	0.0	2.8	27.9	25.6	1.0	0.0
Araliaceae	*Cussonia spicata Thunb.*	P00042a	Roots	DCM	12.1	12.9	0.0	0.0	31.4	4.4	28.2	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Araliaceae	*Cussonia spicata Thunb.*	P00042c	Leaves	MeOH	14.7	7.0	0.0	0.0	91.8	15.6	17.0	1.6
Araliaceae	*Cussonia spicata Thunb.*	P02612b	Leaves	DCM/MeOH (1:1)	1.6	5.8	10.7	2.5	68.0	5.6	70.8	12.2
Poaceae	*Cymbopogon validus*	P12881b	Whole plant	DCM/MeOH (1:1)	98.7	6.2	5.7	5.9	40.8	7.3	97.5	7.0
Rutaceae	*Diosma sp (exact species not identified)*	P02051b	Roots	DCM/MeOH (1:1)	10.0	3.1	0.0	0.0	17.5	0.0	13.8	0.0
Rutaceae	*Diosma sp(exact species not identified)*	P02051a	Roots	DCM	17.4	13.3	0.8	0.4	68.8	14.6	93.3	17.0
Apocynaceae	*Diplorhynchus condylocarpon*	P00940b	Roots	DCM/MeOH (1:1)	0.0	5.6	0.0	0.0	19.3	0.0	4.1	2.9
Sapindaceae	*Dodonaea viscosa Jacq.*	P02291b	Leaves	DCM/MeOH (1:1)	1.5	1.4	5.2	5.1	65.0	14.4	32.5	0.0
Meliaceace	*Ekebergia capensis Sparrm.*	P03111b	Fruits	DCM/MeOH (1:1)	99.4	11.9	0.0	0.0	96.4	20.4	81.3	17.4
Meliaceace	*Ekebergia capensis Sparrm.*	P03112b	Twigs	DCM/MeOH (1:1)	58.0	0.0	0.0	0.0	67.8	6.7	44.0	4.0
Meliaceace	*Ekebergia capensis Sparrm.*	P03111c	Fruits	Aqueous	0.0	0.0	0.0	0.0	10.3	1.2	0.0	0.0
Fabaceae	*Elephantorrhiza elephantina*	P08224c	roots	Aqueous	41.2	20.1	18.4	0.0	29.3	20.3	6.1	0.0
Fabaceae	*Elephantorrhiza elephantina*	P08225c	Leaves	Aqueous	7.4	11.9	0.0	0.9	21.8	7.3	3.5	0.0
Fabaceae	*Elephantorrhiza elephantina (Burch.)*	P08224b	Roots	DCM/MeOH (1:1)	0.0	2.5	0.0	0.0	31.6	2.9	22.6	0.0
Fabaceae	*Elephantorrhiza elephantina (Burch.)*	P08224b	Leaves	DCM/MeOH (1:1)	2.3	0.0	0.0	3.2	27.0	5.6	34.8	0.0
Ebenaceae	*Euclea natalensis A.DC.*	P08226b	Stem bark	DCM/MeOH (1:1)	4.4	0.1	0.0	0.0	53.1	0.0	40.4	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Ebenaceae	*Euclea natalensis A.DC.*	P08227b	Roots	DCM/MeOH (1:1)	98.4	0.0	0.0	0.0	42.7	0.0	79.8	17.1
Ebenaceae	*Euclea undulata Thunb.*	P09984b	Leaves	DCM/MeOH (1:1)	0.0	0.0	0.0	3.7	57.7	6.3	39.2	0.0
Ebenaceae	*Euclea undulata Thunb.*	P09985b	Twigs	DCM/MeOH (1:1)	4.0	0.0	6.8	5.3	34.9	5.2	65.7	0.0
Hyacinthaceae	*Eucomis autumnalis (Mill.) Chitt.*	P01463a	Flowers/Buds	DCM	2.5	0.0	0.0	0.0	95.5	33.4	22.8	2.4
Hyacinthaceae	*Eucomis autumnalis (Mill.) Chitt.*	P01463b	Flowers/Buds	DCM/MeOH (1:1)	7.3	9.1	55.1	0.0	28.8	7.3	2.8	0.0
Hyacinthaceae	*Eucomis autumnalis (Mill.) Chitt.*	P01463c	Flowers/Buds	Aqueous	18.5	19.0	0.0	0.0	15.8	7.8	0.0	0.0
Euphorbiaceae	*Euphorbia heterophylla L.*	P12864b	Whole plant	DCM/MeOH (1:1)	6.6	7.3	0.0	0.7	30.4	6.0	40.8	7.2
Euphorbiaceae	*Euphorbia tirucalli L.*	P00788a	Leaves	DCM	86.5	0.0	0.0	0.0	43.7	8.2	60.3	0.0
Euphorbiaceae	*Euphorbia tirucalli L.*	P00788b	Leaves	DCM/MeOH (1:1)	0.0	0.0	0.0	0.0	22.3	0.0	15.4	2.9
Euphorbiaceae	*Euphorbia tirucalli L.*	P00788c	Leaves	MeOH	18.7	21.3	3.9	5.9	28.7	2.4	0.0	0.0
Flacourtiaceae	*Flacourtia indica (Burm.f.) Merr.*	P00904a	Roots	DCM	0.0	0.9	0.0	0.0	10.6	1.8	9.3	0.0
Flacourtiaceae	*Flacourtia indica (Burm.f.) Merr.*	P00904c	Roots	Aqueous	11.4	4.6	0.0	0.0	10.8	0.0	0.3	0.4
Colchicaceae	*Gloriosa superba L.*	P08215b	Whole plant	DCM/MeOH (1:1)	7.5	9.2	26.7	0.0	24.9	13.1	43.6	11.4
Colchicaceae	*Gloriosa superba L.*	P08215c	whole plant	Aqueous	0.0	17.1	23.4	0.0	12.5	4.5	0.0	0.0
Apocynaceae	*Gomphocarpus fruticosus (L.) Aiton.f.*	P09988b	Fruits	DCM/MeOH(1:1)	1.4	0.0	7.4	0.0	21.7	14.3	5.3	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Apocynaceae	*Gomphocarpus fruticosus (L.) Aiton.f.*	P09989b	Leaves	DCM/MeOH(1:1)	9.1	0.6	2.9	2.9	21.4	11.2	22.7	0.0
Asteraceae	*Helichrysum nudifolium.*	P02847b	Whole plant	DCM/MeOH (1:1)	0.0	10.7	0.0	0.0	99.9	14.0	63.2	3.2
Sapindaceae	*Hippobromus pauciflorus (L.f.) Radlk.*	P12876b	Leaves	DCM/MeOH (1:1)	0.0	0.0	0.0	0.0	32.0	15.4	58.6	0.0
Sapindaceae	*Hippobromus pauciflorus (L.f.) Radlk.*	P12876c	Leaves	Aqueous	13.8	0.8	0.6	0.0	15.4	3.8	0.0	0.0
Sapindaceae	*Hippobromus pauciflorus (L.f.) Radlk.*	P12877b	Twigs	DCM/MeOH (1:1)	4.2	7.5	2.5	0.0	73.2	6.0	90.9	13.1
Clusiaceae	*Hypericum aethiopicum Thunb.*	P02817b	Leaves	DCM/MeOH (1:1)	98.8	75.1	5.9	2.7	100.0	55.2	100.0	32.9
Clusiaceae	*Hypericum aethiopicum Thunb.*	P02817c	Leaves	Aqueous	0.0	4.7	0.0	0.0	14.6	1.7	0.0	5.1
Lamiaceae	*Hyptis pectinata (L.) Poit.*	P02459b	Leaves	DCM/MeOH (1:1)	0.0	2.4	26.7	0.0	37.1	6.3	34.2	0.0
Lamiaceae	*Hyptis pectinata (L.) Poit.*	P02459c	Twigs/Leaves	Aqueous	8.8	0.7	2.2	2.8	31.5	26.9	6.8	0.6
Acanthaceae	*Justicia flava*	P05636b	Whole plant	DCM/MeOH (1:1)	0.0	11.8	0.0	0.0	11.9	0.0	21.0	0.0
Bignoniaceae	*Kigelia africana (Lam.) Benth.*	P00692a	Leaves	DCM	11.5	12.0	0.0	0.0	32.7	3.0	6.3	0.0
Bignoniaceae	*Kigelia africana (Lam.) Benth.*	P00692b	Leaves	DCM/MeOH (1:1)	14.7	4.2	0.0	0.0	9.5	9.2	6.1	0.0
Kirkiaceae	*Kirkia wilmsii Engl.*	P13041c	Leaves	Aqueous	0.0	0.0	0.0	0.0	18.4	7.4	60.9	0.0
Lamiaceae	*Leonotis leonurus (L.) R.Br.*	P03268b	Twigs	DCM/MeOH (1:1)	3.1	12.9	0.0	0.0	48.3	0.0	85.7	7.5
Lamiaceae	*Leonotis leonurus (L.) R.Br.*	P03269b	Leaves	DCM/MeOH (1:1)	32.1	9.1	0.0	0.0	85.0	4.8	81.4	16.3
Lamiaceae	*Leonotis leonurus (L.) R.Br.*	P02414c	roots	Aqueous	7.3	8.2	1.0	1.4	32.3	26.3	7.6	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Lamiaceae	*Leonotis leonurus (L.) R.Br.*	P03268c	Twigs	Aqueous	5.3	0.0	0.0	0.7	12.2	2.4	0.0	0.0
Lamiaceae	*Leonotis leonurus (L.) R.Br.*	P03269c	Leaves	Aqueous	17.1	0.0	0.0	0.0	13.1	3.2	0.0	0.0
Lamiaceae	*Leonotis ocymifolia*	P14867b	Whole plant	DCM/MeOH (1:1)	98.5	14.5	0.0	0.0	35.5	5.1	38.6	0.0
Lamiaceae	*Leonotis ocymifolia*	P00480d	Leaves	Aqueous	6.5	6.8	4.5	6.8	15.8	1.0	0.0	0.0
Lamiaceae	*Leonotis ocymifolia*	P00481d	Fruits	Aqueous	0.0	3.1	0.0	0.0	25.5	9.9	0.0	0.2
Lamiaceae	*Leonotis ocymifolia*	P00482c	Roots	MeOH	0.9	2.5	0.0	0.0	13.6	0.0	1.0	0.0
Lamiaceae	*Leonotis ocymifolia*	P00482d	Roots	Aqueous	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0
Lamiaceae	*Leonotis ocymifolia var. ocymifolia*	P00480a	Leaves	DCM	99.6	12.9	0.0	0.0	54.4	10.2	74.4	9.3
Lamiaceae	*Leonotis ocymifolia var. ocymifolia*	P00480b	Leaves	DCM/MeOH (1:1)	99.1	22.3	0.0	0.0	60.0	8.9	81.0	0.5
Lamiaceae	*Leonotis ocymifolia var. ocymifolia*	P00480c	Leaves	MeOH	19.6	26.6	0.0	0.0	46.4	10.7	66.0	4.4
Lamiaceae	*Leonotis ocymifolia var. ocymifolia*	P00481a	Fruits	DCM	13.2	12.9	0.0	0.0	49.4	8.4	35.7	3.4
Lamiaceae	*Leonotis ocymifolia var. ocymifolia*	P00481b	Fruits	DCM/MeOH (1:1)	0.0	0.0	0.0	0.0	36.1	3.7	6.9	0.0
Lamiaceae	*Leonotis ocymifolia var. ocymifolia*	P00481c	Fruits	MeOH	0.1	0.4	0.6	0.0	20.6	8.0	5.8	0.0
Lamiaceae	*Leonotis ocymifolia var. ocymifolia*	P00482a	Roots	DCM	20.3	10.6	0.0	0.0	36.7	0.0	21.7	0.0
Lamiaceae	*Leonotis ocymifolia var. ocymifolia*	P00482b	Roots	DCM/MeOH (1:1)	14.0	4.8	0.0	0.0	5.4	0.0	9.1	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Lamiaceae	*Leucas martinicensis (L.) R.Br.*	P12950b	Whole plant	DCM/MeOH (1:1)	90.7	19.2	3.6	11.8	53.1	12.5	83.8	10.2
Lamiaceae	*Leucas martinicensis (L.) R.Br.*	P12950c	whole plant	Aqueous	8.9	6.3	5.9	8.3	29.9	19.9	1.7	0.0
Rutaceae	*Macrostylis squarrosa*	P02402b	Stem bark	DCM/MeOH (1:1)	8.0	7.0	0.0	0.0	41.3	0.0	78.6	12.6
Maesaceae	*Maesa lanceolata Forssk.*	P12946c	Twigs	Aqueous	0.0	0.0	7.8	5.7	26.5	18.9	5.7	4.1
Celastraceae	*Maytenus senegalensis (Lam.) Exell.*	P00690a	Roots	DCM	13.6	2.0	0.0	0.0	27.7	7.8	35.2	0.0
Celastraceae	*Maytenus senegalensis (Lam.) Exell.*	P00693a	Stem bark	DCM	0.0	6.0	0.0	0.0	24.1	0.0	25.1	0.5
Celastraceae	*Maytenus senegalensis (Lam.) Exell.*	P00693b	Stem bark	DCM/MeOH (1:1)	10.5	10.5	0.0	0.0	19.5	0.0	4.6	0.0
Celastraceae	*Maytenus senegalensis (Lam.) Exell.*	P00693d	Stem bark	Aqueous	0.0	1.5	1.9	0.0	10.0	0.0	0.0	0.0
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00151b	Leaves	DCM/MeOH (1:1)	0.0	12.4	0.0	0.0	46.0	4.9	23.7	18.3
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00152a	Stem bark	DCM	17.7	13.4	3.4	0.0	83.4	17.0	25.9	5.2
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00152b	Stem bark	DCM/MeOH (1:1)	0.0	1.6	0.0	0.0	53.7	9.5	11.3	5.8
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00153a	Roots	DCM	0.8	5.8	0.0	0.0	98.7	30.2	42.4	0.3
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00153b	Roots	DCM/MeOH (1:1)	6.5	6.8	0.0	0.0	82.0	15.9	17.0	0.0
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00153c	Roots	MeOH	0.0	1.9	0.0	0.0	28.7	2.2	5.4	0.0
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00151c	Leaves	MeOH	4.2	7.0	0.0	0.0	18.8	3.6	13.8	9.4
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00152c	Stem bark	MeOH	0.0	1.6	3.7	2.5	38.7	14.3	6.1	0.0
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00151a	Leaves	DCM	2.8	9.4	0.0	0.0	41.2	0.0	8.3	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00151d	Leaves	Aqueous	2.7	0.0	9.1	9.3	17.0	0.2	0.0	0.0
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00152d	Stem bark	Aqueous	0.0	0.0	13.1	2.9	16.1	10.9	0.0	0.0
Celastraceae	*Maytenus undata (Thunb.) Blakelock*	P00153d	Roots	Aqueous	8.6	0.0	14.2	3.6	20.4	12.6	0.0	0.0
Cucurbitaceae	*Momordica balsamina L.*	P04038b	Stem bark	DCM/MeOH (1:1)	2.2	0.5	0.0	0.0	33.6	2.6	83.4	17.0
Cucurbitaceae	*Momordica balsamina L.*	P04039b	Leaves	DCM/MeOH (1:1)	21.2	22.7	0.0	0.0	49.2	7.8	64.7	4.5
Lamiaceae	*Ocimum americanum L.*	P12866b	Whole plant	DCM/MeOH (1:1)	0.0	4.3	0.0	0.0	30.2	2.5	100.1	0.1
Oleaceae	*Olea europaea L.*	P12848b	Leaves	DCM/MeOH (1:1)	55.2	0.0	14.5	5.8	52.0	8.4	46.3	0.0
Oleaceae	*Olea europaea L.*	P12849b	Twigs	DCM/MeOH (1:1)	46.4	0.0	9.9	3.9	43.8	13.0	50.5	0.0
Asteraceae	*Osteospermum imbricatum L.*	P02640b	Stem bark	DCM/MeOH (1:1)	7.4	12.9	3.5	1.8	51.4	2.6	92.9	3.0
Chrysobalanaceae	*Parinari curatellifolia Planch. ex Benth.*	P00253a	Leaves	DCM	0.0	3.5	0.0	0.0	66.9	21.7	53.0	0.0
Chrysobalanaceae	*Parinari curatellifolia Planch. ex Benth.*	P00253b	Leaves	DCM/MeOH (1:1)	18.7	4.7	0.0	0.0	26.0	0.0	9.1	0.0
Chrysobalanaceae	*Parinari curatellifolia Planch. ex Benth.*	P00253c	Leaves	MeOH	0.0	1.2	0.0	0.0	11.3	0.0	0.0	0.0
Chrysobalanaceae	*Parinari curatellifolia Planch. ex Benth.*	P00253d	Leaves	Aqueous	0.0	7.8	0.0	0.0	17.4	6.7	3.3	0.0
Chrysobalanaceae	*Parinari curatellifolia Planch. ex Benth.*	P00254b	Roots	DCM/MeOH (1:1)	0.0	11.8	0.0	0.0	26.9	11.0	22.7	0.0
Chrysobalanaceae	*Parinari curatellifolia Planch. ex Benth.*	P00254c	Roots	MeOH	13.7	8.5	0.0	0.0	23.3	13.6	11.7	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Chrysobalanaceae	*Parinari curatellifolia Planch. ex Benth.*	P00254d	Roots	Aqueous	8.9	6.6	0.0	0.0	13.9	2.4	0.0	1.7
Fabaceae	*Parkinsonia aculeata L.*	P09990b	Twigs	DCM/MeOH (1:1)	5.8	1.6	0.0	2.8	26.3	3.0	32.3	0.0
Gentianaceae	*Pelargonium alchemilloides (L.) L'Hér.*	P08205b	Whole plant	DCM/MeOH (1:1)	6.8	11.4	0.5	0.0	33.0	8.1	87.3	10.4
Asteraceae	*Pentzia globosa Less.*	P01514a	Leaves	DCM	91.3	0.0	6.9	0.0	84.2	1.9	62.5	7.9
Asteraceae	*Pentzia globosa Less.*	P01514b	Leaves	DCM/MeOH (1:1)	0.0	0.0	0.0	3.3	26.3	0.3	39.2	0.0
Asteraceae	*Pentzia globosa Less.*	P01514c	Leaves	Aqueous	9.2	0.0	2.4	0.0	25.2	4.1	5.7	14.9
Asteraceae	*Pentzia globosa Less.*	P01516a	Roots	DCM	99.1	6.5	9.8	9.4	63.2	18.4	96.0	20.8
Asteraceae	*Pentzia globosa Less.*	P01516b	Roots	DCM/MeOH (1:1)	98.0	3.4	4.1	1.0	35.9	0.0	61.5	7.2
Asteraceae	*Pentzia globosa Less.*	P01517a	Stem bark	DCM	98.9	0.0	7.7	0.0	33.9	12.2	76.1	5.3
Asteraceae	*Pentzia globosa Less.*	P01517b	Stem bark	DCM/MeOH (1:1)	99.6	0.0	0.0	0.0	16.0	0.0	29.6	0.0
Fabaceae	*Piliostigma thonningii*	P18548c	Fruits	Aqueous	16.6	2.8	1.0	0.0	20.0	5.2	0.0	0.0
Fabaceae	*Piliostigma thonningii*	P18547c	Leaves	Aqueous	24.3	26.5	0.0	0.0	20.8	8.3	5.8	0.0
Fabaceae	*Piliostigma thonningii*	P18549c	Twigs	Aqueous	18.9	6.4	3.5	0.0	22.4	2.7	0.0	0.0
Pittosporaceae	*Pittosporum viridiflorum Sims*	P00213b	Whole plant	DCM/MeOH (1:1)	49.8	10.7	0.0	0.0	28.6	11.2	59.4	3.2
Pittosporaceae	*Pittosporum viridiflorum Sims*	P00213c	Whole plant	MeOH	0.0	3.9	0.0	2.3	14.8	8.9	14.6	0.0
Pittosporaceae	*Pittosporum viridiflorum Sims*	P00215a	Leaves	DCM	7.0	5.5	0.0	0.0	31.0	6.8	25.2	2.8

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Pittosporaceae	*Pittosporum viridiflorum Sims*	P00215b	Leaves	DCM/MeOH (1:1)	1.4	0.1	0.0	0.0	4.4	0.0	0.0	0.0
Pittosporaceae	*Pittosporum viridiflorum Sims*	P00215c	Leaves	MeOH	0.0	4.9	0.0	0.0	23.3	9.0	0.0	0.0
Plantaginaceae	*Plantago major L.*	P01571b	Whole plant	DCM/MeOH (1:1)	0.0	0.0	0.0	0.0	17.8	5.4	16.5	0.9
Plumbaginaceae	*Plumbago zeylanica L.*	P00630a	Roots	DCM	20.5	19.4	0.0	0.0	19.5	0.0	16.1	0.0
Plumbaginaceae	*Plumbago zeylanica L.*	P00630b	Roots	DCM/MeOH (1:1)	10.9	9.0	0.0	0.0	7.5	0.0	18.7	0.0
Plumbaginaceae	*Plumbago zeylanica L.*	P00630c	Roots	MeOH	18.7	8.7	0.0	0.0	11.9	0.0	0.0	10.5
Plumbaginaceae	*Plumbago zeylanica L.*	P00631b	Leaves	DCM/MeOH (1:1)	97.4	7.8	0.0	0.0	32.1	2.2	31.6	8.1
Illecebraceae	*Pollichia campestris Aiton*	P00390b	Leaves	DCM/MeOH (1:1)	0.0	8.0	6.1	2.7	24.6	2.8	22.5	0.0
Illecebraceae	*Pollichia campestris Aiton*	P00390c	Leaves	MeOH	18.0	9.4	0.0	0.0	18.3	5.9	0.0	0.0
Illecebraceae	*Pollichia campestris Aiton*	P00391a	Fruits	DCM	4.0	0.0	0.0	0.0	41.7	6.3	57.7	0.0
Illecebraceae	*Pollichia campestris Aiton*	P00391b	Fruits	DCM/MeOH (1:1)	3.5	8.1	0.0	0.0	21.8	5.8	6.9	0.0
Illecebraceae	*Pollichia campestris Aiton*	P00391c	Leaves	MeOH	12.7	16.0	0.0	0.0	27.1	2.2	2.6	0.0
Illecebraceae	*Pollichia campestris Aiton*	P03065b	Whole plant	DCM/MeOH (1:1)	9.3	9.7	0.0	0.0	51.0	0.0	83.8	8.5
Illecebraceae	*Pollichia campestris Aiton*	P00390d	Leaves	Aqueous	0.0	0.0	0.0	0.0	16.1	6.3	0.0	0.0
Illecebraceae	*Pollichia campestris Aiton*	P03065c	whole plant	Aqueous	0.0	0.0	0.0	0.0	14.8	0.0	0.0	0.3
Illecebraceae	*Pollichia campestris Aiton*	P03318c	Twigs	Aqueous	19.4	0.0	0.0	0.6	17.1	1.9	0.0	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Asteraceae	*Psiadia punctulata*	P00819b	Leaves	DCM/MeOH (1:1)	18.8	3.2	0.0	0.0	47.7	8.2	34.9	3.7
Asteraceae	*Psiadia punctulata*	P02527b	Whole plant	DCM/MeOH (1:1)	24.0	0.0	10.0	3.6	47.9	8.3	48.7	0.0
Ptaeroxylaceae	*Ptaeroxylon obliquum (Thunb.) Radlk.*	P01842a	Roots	DCM	10.5	12.9	9.0	4.7	35.3	0.9	44.4	1.0
Ptaeroxylaceae	*Ptaeroxylon obliquum (Thunb.) Radlk.*	P01842b	Roots	DCM/MeOH (1:1)	4.9	11.8	9.6	0.0	44.7	12.4	56.7	4.5
Ptaeroxylaceae	*Ptaeroxylon obliquum (Thunb.) Radlk.*	P01870a	Leaves	DCM	99.7	0.0	0.0	0.0	55.0	5.5	41.3	5.4
Ptaeroxylaceae	*Ptaeroxylon obliquum (Thunb.) Radlk.*	P01870b	Leaves	DCM/MeOH (1:1)	0.0	0.0	0.0	0.0	19.1	2.4	24.2	0.0
Ptaeroxylaceae	*Ptaeroxylon obliquum (Thunb.) Radlk.*	P01890a	Stem bark	DCM	10.1	0.0	0.0	0.0	52.7	1.4	79.8	17.3
Ptaeroxylaceae	*Ptaeroxylon obliquum (Thunb.) Radlk.*	P01890b	Stem bark	DCM/MeOH (1:1)	30.1	4.6	0.0	0.0	40.3	4.2	65.4	12.8
Fabaceae	*Pterocarpus angolensis DC.*	P00304b	Stem bark	DCM/MeOH (1:1)	0.0	6.6	0.0	0.0	17.4	8.8	0.2	0.0
Fabaceae	*Pterocarpus angolensis DC.*	P00304c	Stem bark	MeOH	6.5	6.4	0.0	0.0	16.2	6.2	0.7	1.0
Fabaceae	*Pterocarpus angolensis DC.*	P00305b	Roots	DCM/MeOH (1:1)	18.3	0.0	0.0	0.0	20.9	5.3	16.8	0.0
Fabaceae	*Pterocarpus angolensis DC.*	P00305c	Roots	MeOH	16.3	1.6	0.0	0.0	11.8	0.4	0.0	0.0
Apocynaceae	*Rauvolfia caffra Sond.*	P00734a	Roots	DCM	99.7	7.6	0.0	3.1	2.7	0.4	44.6	0.0
Apocynaceae	*Rauvolfia caffra Sond.*	P00734b	Fruits	DCM/MeOH (1:1)	4.9	9.0	0.0	0.0	24.0	1.2	19.9	4.2
Apocynaceae	*Rauvolfia caffra Sond.*	P00735b	Roots	DCM/MeOH (1:1)	6.5	0.0	0.0	0.0	15.0	1.5	9.5	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Apocynaceae	*Rauvolfia caffra Sond.*	P00735d	Roots	Aqueous	5.7	6.7	0.0	0.0	13.0	0.0	0.0	0.0
Apocynaceae	*Rauvolfia caffra Sond.*	P00735a	Fruits	DCM	13.1	16.0	0.0	0.6	19.7	7.6	15.4	0.0
Euphorbiaceae	*Ricinus communis L. var. communis*	P02300b	Leaves	DCM/MeOH (1:1)	0.0	4.4	0.0	0.0	62.7	0.8	55.9	9.5
Euphorbiaceae	*Ricinus communis L. var. communis*	P02311b	Fruits	DCM/MeOH (1:1)	5.3	1.4	0.0	0.0	6.9	0.0	12.9	0.0
Euphorbiaceae	*Ricinus communis L. var. communis*	P02355b	Stem bark	DCM/MeOH (1:1)	0.0	0.0	0.0	0.0	46.4	0.0	78.7	13.8
Euphorbiaceae	*Ricinus communis L. var. communis*	P02300c	Leaves	Aqueous	6.9	2.3	10.9	0.9	34.6	22.0	2.4	6.7
Euphorbiaceae	*Ricinus communis L. var. communis*	P02311c	Fruits	Aqueous	8.1	5.1	9.8	0.1	40.1	27.2	6.9	0.0
Euphorbiaceae	*Ricinus communis L. var. communis*	P02355c	Stem bark	Aqueous	4.9	9.8	0.0	0.0	38.1	27.6	7.0	0.0
Polygonaceae	*Rumex crispus L.*	P01689b	Roots	DCM/MeOH (1:1)	0.0	0.0	0.0	0.0	20.1	7.9	21.0	4.8
Polygonaceae	*Rumex crispus L.*	P01634a	Leaves	DCM	2.1	0.0	0.0	0.0	11.6	3.7	9.7	2.4
Polygonaceae	*Rumex crispus L.*	P01634b	Leaves	DCM/MeOH (1:1)	0.0	3.6	0.0	0.0	18.7	0.4	0.1	0.0
Polygonaceae	*Rumex crispus L.*	P01689a	Roots	DCM	0.9	3.6	0.0	0.0	28.0	5.4	55.3	5.0
Polygonaceae	*Rumex sagittatus Thunb.*	P12875b	Whole plant	DCM/MeOH (1:1)	6.7	0.0	4.0	0.0	18.5	3.6	38.4	0.0
Lamiaceae	*Salvia repens Burch. ex Benth.*	P08214b	Whole plant	DCM/MeOH(1:1)	21.0	6.7	2.7	1.4	101.3	35.2	62.6	2.1
Goodeniaceae	*Scaevola plumieri (L.) Vahl*	P08206b	Twigs	DCM/MeOH (1:1)	6.5	10.0	0.0	0.7	26.8	5.4	22.2	0.0
Araliaceae	*Schefflera umbellifera (Sond.) Baill.*	P00245b	Leaves	DCM/MeOH (1:1)	22.6	7.8	0.0	0.0	68.7	16.4	64.1	16.4

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Araliaceae	*Schefflera umbellifera (Sond.) Baill.*	P00245c	Leaves	MeOH	8.0	11.4	0.0	0.0	39.0	10.6	23.5	4.8
Araliaceae	*Schefflera umbellifera (Sond.) Baill.*	P00246a	Roots	DCM	0.0	10.7	0.0	0.0	80.1	16.0	25.7	9.4
Araliaceae	*Schefflera umbellifera (Sond.) Baill.*	P00246b	Roots	DCM/MeOH (1:1)	0.0	16.0	0.0	0.0	71.1	15.3	39.1	6.2
Araliaceae	*Schefflera umbellifera (Sond.) Baill.*	P00248a	Stem bark	DCM	4.5	0.0	8.5	0.0	57.0	9.2	45.7	4.6
Araliaceae	*Schefflera umbellifera (Sond.) Baill.*	P00248b	Stem bark	DCM/MeOH (1:1)	0.0	0.0	0.0	0.0	49.0	12.3	28.0	0.0
Araliaceae	*Schefflera umbellifera (Sond.) Baill.*	P00248c	Stem bark	MeOH	10.4	6.0	0.0	0.0	28.0	6.3	2.9	0.0
Araliaceae	*Schefflera umbellifera (Sond.) Baill.*	P00248d	Stem bark	Aqueous	13.2	14.7	8.7	10.4	17.0	9.2	0.0	0.0
Asteraceae	*Senecio oxyriifolius DC.*	P08209b	Whole plant	DCM/MeOH(1:1)	0.0	7.3	9.0	0.0	32.4	10.1	51.3	5.7
Fabaceae	*Senna didymobotrya*	P08219b	Leaves	DCM/MeOH (1:1)	13.2	0.4	0.0	2.9	30.8	1.2	35.5	2.6
Fabaceae	*Senna didymobotrya*	P08220b	Twigs	DCM/MeOH (1:1)	21.6	17.1	0.0	0.0	39.4	5.0	54.0	9.5
Fabaceae	*Senna didymobotrya*	P08221b	Pods	DCM/MeOH (1:1)	9.6	1.8	0.0	0.0	29.1	7.7	34.5	3.3
Fabaceae	*Senna didymobotrya*	P08220c	Twigs	Aqueous	11.0	7.7	10.8	1.9	30.1	14.7	0.0	0.0
Fabaceae	*Senna didymobotrya*	P08219c	Leaves	Aqueous	3.2	3.3	0.0	0.0	12.9	3.1	0.0	0.0
Fabaceae	*Senna petersiana (Bolle) Lock*	P18564c	Leaves	Aqueous	1.5	0.0	3.5	3.8	28.0	23.2	0.0	1.1
Fabaceae	*Senna petersiana (Bolle) Lock*	P18565c	Twigs	Aqueous	0.0	1.7	14.8	7.5	30.6	20.8	0.0	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Poaceae	*Setaria megaphylla*	P12880b	Whole plant	DCM/MeOH (1:1)	18.1	2.0	3.8	7.4	34.1	4.0	99.8	19.0
Asteraceae	*Spilanthes mauritiana (Pers.) DC.*	P00274a	Stem bark	DCM	10.9	10.0	0.0	0.0	60.0	8.5	70.6	0.8
Asteraceae	*Spilanthes mauritiana (Pers.) DC.*	P00274b	Stem bark	DCM/MeOH (1:1)	2.0	11.3	0.1	0.0	35.4	9.0	29.8	1.3
Asteraceae	*Spilanthes mauritiana (Pers.) DC.*	P00274c	Stem bark	MeOH	4.3	0.0	0.0	0.0	17.2	0.6	0.0	0.0
Myrtaceae	*Syzigium cordatum*	P18315c	Twigs	Aqueous	13.1	16.4	2.5	8.2	15.3	6.1	16.9	0.0
Myrtaceae	*Syzigium cordatum*	P18315b	Twigs	DCM/MeOH (1:1)	0.0	0.9	0.0	0.0	51.1	16.4	54.0	10.8
Myrtaceae	*Syzigium cordatum*	P18316b	Leaves	DCM/MeOH (1:1)	0.0	4.7	0.0	0.0	56.5	11.6	41.6	0.0
Myrtaceae	*Syzigium cordatum*	P18316c	Leaves	Aqueous	0.0	0.0	0.0	0.0	9.3	0.0	84.0	0.0
Asteraceae	*Tarchonanthus camphoratus L.*	P01089a	Leaves	DCM	85.8	0.0	7.4	0.9	75.2	15.8	76.1	1.6
Asteraceae	*Tarchonanthus camphoratus L.*	P01089b	Leaves	DCM/MeOH (1:1)	14.2	0.0	0.0	0.0	62.0	7.8	41.7	0.0
Asteraceae	*Tarchonanthus camphoratus L.*	P01154b	Roots	DCM/MeOH (1:1)	95.4	0.0	0.0	0.0	52.3	1.4	17.5	3.9
Asteraceae	*Tarchonanthus camphoratus L.*	P02554b	Whole plant	DCM/MeOH (1:1)	98.9	63.8	6.0	1.1	100.0	24.8	49.3	0.0
Lamiaceae	*Tetradenia riparia (Hochst.) Codd*	P00741a	Leaves	DCM	0.0	0.0	0.0	2.4	36.4	0.0	37.8	0.0
Lamiaceae	*Tetradenia riparia (Hochst.) Codd*	P00741b	Leaves	DCM/MeOH (1:1)	6.4	0.0	1.6	2.0	16.0	0.0	7.4	7.1
Lamiaceae	*Tetradenia riparia (Hochst.) Codd*	P00741d	Leaves	Aqueous	0.0	6.8	4.2	6.7	9.5	0.0	0.0	0.0
Meliaceace	*Trichilia emetica Vahl subsp. emetica*	P02470c	Twigs/Leaves	Aqueous	13.0	6.0	0.0	0.0	15.2	6.8	0.8	0.0
Meliaceace	*Turraea floribunda Hochst.*	P15192c	Leaves	Aqueous	23.9	27.5	0.0	1.5	20.7	4.9	1.2	3.7

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Rubiaceae	*Vangueria infausta*	P02497b	Fruits	DCM/MeOH (1:1)	2.7	4.2	0.0	0.0	33.2	0.0	32.6	0.0
Asteraceae	*Vernonia colorata*	P18569c	Twigs	Aqueous	0.0	6.0	5.7	8.5	29.4	19.2	1.5	0.0
Asteraceae	*Vernonia fastigiata Oliv. & Hiern*	P00393b	Leaves	DCM/MeOH (1:1)	58.3	3.7	0.0	0.0	28.7	0.0	30.3	0.0
Asteraceae	*Vernonia fastigiata Oliv. & Hiern*	P00393c	Leaves	MeOH	10.0	10.9	0.0	0.0	23.9	8.1	21.1	0.0
Asteraceae	*Vernonia hirsuta*	P02834b	Whole plant	DCM/MeOH (1:1)	99.1	4.9	0.0	0.0	80.6	6.2	45.8	0.0
Asteraceae	*Vernonia myriantha Hook.f.*	P00170a	Roots	DCM	0.0	0.0	0.0	0.0	19.0	0.0	20.9	0.0
Asteraceae	*Vernonia myriantha Hook.f.*	P00170b	Roots	DCM/MeOH (1:1)	6.3	14.6	0.0	0.0	30.6	9.9	16.1	4.9
Asteraceae	*Vernonia myriantha Hook.f.*	P00170d	Roots	Aqueous	7.2	7.7	0.0	0.0	17.2	6.1	16.1	7.5
Asteraceae	*Vernonia myriantha Hook.f.*	P00171a	Leaves	DCM	4.7	10.1	0.0	0.0	33.8	10.1	35.1	1.4
Asteraceae	*Vernonia myriantha Hook.f.*	P00171b	Leaves	DCM/MeOH (1:1)	6.4	9.9	0.0	0.0	22.7	9.4	11.3	0.0
Asteraceae	*Vernonia myriantha Hook.f.*	P00170c	Roots	MeOH	9.7	0.0	0.0	0.0	22.3	3.6	0.0	0.0
Asteraceae	*Vernonia natalensis*	P08212b	Whole plant	DCM/MeOH (1:1)	99.9	4.5	1.1	2.3	45.6	7.8	56.7	10.5
Asteraceae	*Vernonia oligocephala*	P00989b	Roots	DCM/MeOH (1:1)	0.0	0.0	2.7	0.0	13.5	1.4	24.2	0.0
Asteraceae	*Vernonia oligocephala*	P01015a	Leaves	DCM	99.0	78.0	23.1	0.0	59.5	3.7	88.7	11.0
Asteraceae	*Vernonia oligocephala*	P01015b	Leaves	DCM/MeOH (1:1)	99.3	0.0	0.0	0.0	20.9	6.3	29.4	0.0
Olacaceae	*Ximenia caffra Sond. var. caffra*	P00351a	Leaves	DCM	0.0	6.8	0.0	0.0	30.6	6.4	11.7	0.0

					T. b. rhodesience		*T. cruzi*		*L. donovani*		*P. falciparum*	
					Growth Inhibition (%)		**Growth Inhibition (%)**		**Growth Inhibition (%)**		**Growth Inhibition (%)**	
Family	***Species***	**Voucher number**	**Plant part**	**Extract Type**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**	**9.7 µg/ml**	**1.6 µg/ml**
Olacaceae	*Ximenia caffra Sond. var. caffra*	P00351b	Leaves	DCM/MeOH (1:1)	0.0	6.6	0.0	0.0	16.0	10.2	6.0	0.0
Olacaceae	*Ximenia caffra Sond. var. caffra*	P00351d	Leaves	Aqueous	8.2	10.5	4.6	4.3	17.2	0.0	0.0	0.0
Olacaceae	*Ximenia caffra Sond. var. caffra*	P00352a	Roots	DCM	4.5	12.3	0.0	0.0	21.4	4.0	1.3	0.0
Olacaceae	*Ximenia caffra Sond. var. caffra*	P00352b	Roots	DCM/MeOH (1:1)	1.3	2.4	0.0	0.0	15.8	8.1	0.0	0.0
Olacaceae	*Ximenia caffra Sond. var. caffra*	P00352c	Roots	MeOH	1.4	6.9	0.0	0.0	18.8	3.6	0.0	0.0
Apocynaceae	*Xysmalobium undulatum (L.) Aiton.f.*	P12869b	Whole plant	DCM/MeOH(1:1)	8.3	9.7	0.0	0.0	29.6	0.0	28.2	0.5
Cucurbitaceae	*Zehneria scabra*	P08210b	Whole plant	DCM/MeOH (1:1)	4.7	8.3	9.3	5.9	21.5	0.0	20.8	5.4
Cucurbitaceae	*Zehneria scabra*	P08210c	whole plant	Aqueous	27.6	0.0	0.0	3.8	17.4	12.7	0.0	4.2

Table 2.2 Antiprotozoal *in vitro* activity (IC_{50} values in µg/mL) of 43 plant extracts against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. Extracts were considered "potential hits" when they inhibited one or more of the parasites at 9.7 µg/mL in a preliminary first screen (Appendix 1, Table 2.1). The positive controls melarsoprol, benznidazol, miltefosine, chloroquine and podophyllotoxin were tested likewise. Tests were done twice in duplicate.

Plant name	Plant part	Bioprospecting no.	Extract Type	*T. b. rhodesiense*	*T. cruzi*	*L. donovani*	*P. falciparum*	Cytotoxicity
Agathosma apiculata	Whole plant	P09995b	DCM/MeOH (1:1)	11.10	41.50	16.00	0.21	42.90
Agathosma puberula.	Roots	P02011a	DCM	32.20	28.90	15.10	8.53	57.90
Alepidea amatymbica	Whole plant	P02873b	DCM/MeOH (1:1)	20.30	73.10	12.10	3.70	52.70
Artabotrys monteiroae	Leaves	P18314b	DCM/MeOH (1:1)	10.30	41.30	16.60	8.79	40.90
Artemisia afra	Leaves	P00484b	DCM/MeOH (1:1)	21.90	54.50	8.80	7.50	15.80
Artemisia afra	Leaves	P00484a	DCM	9.60	27.60	5.68	6.22	21.50
Artemisia afra	Leaves	P00484c	MeOH	15.90	41.80	15.10	13.30	47.40
Asystasia gangetica	Leaves	P05623b	DCM/MeOH (1:1)	13.20	74.70	12.40	4.20	15.90
Catha edulis	Roots	P00469a	DCM	14.20	19.10	7.65	4.91	17.80
Conyza albida Spreng.	Whole plant	P12954b	DCM/MeOH (1:1)	18.80	38.30	16.20	5.79	40.80
Conyza podocephala	Whole plant	P03063b	DCM/MeOH (1:1)	13.90	47.20	15.80	5.45	51.60
Conyza scabrida	Leaves	P03170b	DCM/MeOH (1:1)	30.00	49.40	6.65	6.66	48.10
Croton menyhartii	Leaves	P12951b	DCM/MeOH (1:1)	11.70	33.30	15.80	2.63	46.40
Croton menyhartii	Twigs	P12952b	DCM/MeOH (1:1)	11.30	41.10	15.90	2.88	45.40
Croton menyhartii	Whole plant	P14867b	DCM/MeOH (1:1)	8.76	38.60	16.30	10.80	51.10

Plant name	Plant part	Bioprospecting no.	Extract Type	*T. b. rhodesiense*	*T. cruzi*	*L. donovani*	*P. falciparum*	Cytotoxicity
Cymbopogon validus	Whole plant	P12881b	DCM/MeOH (1:1)	11.90	47.10	17.90	6.67	48.70
Ekebergia capensis	Fruits	P03111b	DCM/MeOH (1:1)	11.50	24.40	4.80	3.50	9.90
Ekebergia capensis	Twigs	P03112b	DCM/MeOH (1:1)	15.60	46.40	15.90	13.30	55.40
Euclea natalensis	Roots	P08227b	DCM/MeOH (1:1)	28.10	43.70	14.80	7.59	57.90
Eucomis autumnalis	Flowers/Buds	P01463a	DCM	37.10	29.70	7.62	22.10	51.30
Helichrysum nudifolium	Whole plant	P02847b	DCM/MeOH (1:1)	33.10	43.90	15.30	9.36	47.70
Hypericum aethiopicum	Leaves	P02817b	DCM/MeOH (1:1)	4.47	18.10	4.74	2.35	15.20
Leonotis leonurus	Leaves	P03269b	DCM/MeOH (1:1)	14.70	50.10	4.70	2.90	14.10
Leonotis ocymifolia	Leaves	P00480a	DCM	9.10	63.50	18.50	2.70	22.10
Leonotis ocymifolia	Leaves	P00480b	DCM/MeOH (1:1)	9.70	91.20	13.30	4.50	18.20
Maytenus undata	Roots	P00153a	DCM	35.20	28.40	5.58	8.53	52.40
Pentzia globosa	Roots	P01516a	DCM	5.76	31.30	14.40	4.27	50.20
Pentzia globosa	Stem bark	P01517a	DCM	6.32	46.50	22.00	6.04	63.90
Pentzia globosa	*Roots*	P01516b	DCM/MeOH (1:1)	6.69	40.60	17.50	6.89	54.20
Pentzia globosa	Stem bark	P01517b	DCM/MeOH (1:1)	15.20	55.60	49.50	9.14	>100.00
Plumbago zeylanica	Leaves	P00631b	DCM/MeOH (1:1)	13.00	54.50	17.80	12.40	14.40
Ptaeroxylon obliquum	Leaves	P01870a	DCM	11.30	41.50	17.20	10.90	46.30
Rauvolfia caffra	Roots	P00734a	DCM	17.90	41.10	15.50	8.44	46.60
Salvia repens	Whole plant	P08214b	DCM/MeOH (1:1)	10.80	36.20	5.36	7.65	41.50
Schefflera umbellifera	Roots	P00246a	DCM	20.90	57.60	5.03	2.70	13.90

Plant name	Plant part	Bioprospecting no.	Extract Type	*T. b. rhodesiense*	*T. cruzi*	*L. donovani*	*P. falciparum*	Cytotoxicity
Schefflera umbellifera	Roots	P00246b	DCM/MeOH (1:1)	30.20	99.50	14.10	7.70	48.30
Setaria megaphylla	Whole plant	P12880b	DCM/MeOH (1:1)	16.80	43.90	16.90	4.44	48.10
Tarchonanthus camphoratus	Roots	P01154b	DCM/MeOH (1:1)	13.40	42.90	16.30	17.70	31.90
Tarchonanthus camphoratus	Whole plant	P02554b	DCM/MeOH (1:1)	3.93	18.60	4.86	6.23	16.10
Vernonia hirsuta	Whole plant	P02834b	DCM/MeOH (1:1)	18.10	43.00	16.20	10.20	31.60
Vernonia natalensis	Whole plant	P08212b	DCM/MeOH (1:1)	12.60	39.30	17.20	8.53	38.60
Vernonia oligocephala	Leaves	P01015a	DCM	4.67	14.30	16.10	7.69	6.54
Vernonia oligocephala	Leaves	P01015b	DCM/MeOH (1:1)	10.90	44.50	19.90	9.51	22.30
Melarsoprol				0.004				
Benznidazole					0.48			
Miltefosine						0.24		
Chloroquine							0.052	
Podophyllotoxin								0.003

Table 4.1 Antiprotozoal activity of 60 South African Medicinal plants species against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *P. falciparum*. The activity was expressed as percentage (%) growth inhibition. Plants extracts showing more than 95% growth inhibition at 10 µg/ml were considered potential "hits". The tests were done twice and in duplicate.

					Percentage (%) growth inhibition							
					T. b. rhodesiense		***T cruzi***		***L. donovani***		***P. falciparum***	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Fabaceae	***Abrus precatorius africanus***	Whole plant	DCM/MeOH (1:1)	P06218B	100.0	100.0	44.0	16.7	75.5	16.1	97.8	37.0
Fabaceae	***Acacia caffra***	Leaves	DCM/MeOH (1:1)	P00855B	3.0	0.0	42.3	17.6	33.5	8.3	19.9	0.3
Fabaceae	***Acacia caffra***	Roots	DCM/MeOH (1:1)	P00866B	93.9	0.0	46.1	25.7	34.7	8.9	55.0	7.0
Fabaceae	***Acacia erioloba***	Roots	DCM/MeOH (1:1)	P08470B	18.6	6.4	48.9	18.8	50.2	26.3	61.4	72.6
Euphorbiaceae	***Acalypha peduncularis.***	leaves	DCM/MeOH (1:1)	P02855B	98.8	0.0	37.2	8.5	60.2	0.0	77.7	19.2
Asparagaceae	***Asparagus virgatus***	Leaves	DCM/MeOH (1:1)	P00558B	10.3	0.0	47.6	15.7	0.0	0.0	36.5	0.0
Lamiaceae	***Becium obovatum***	Roots	DCM/MeOH (1:1)	P04123B	49.4	11.9	37.5	3.9	97.5	5.7	99.3	38.2
Scrophulariaceae	***Bowkeria cymosa***	leaves	DCM/MeOH (1:1)	P11785B	46.1	17.6	59.8	26.2	94.1	31.2	87.0	27.8
Scrophulariaceae	***Bowkeria cymosa***	Twigs	DCM/MeOH (1:1)	P11786B	14.0	4.6	36.9	8.4	47.5	24.3	60.1	7.6
Scrophulariaceae	***Bowkeria cymosa***	Roots	DCM/MeOH (1:1)	P11787B	17.1	0.0	53.0	15.2	39.9	17.0	80.7	14.6
Scrophulariaceae	***Bowkeria cymosa***	stems	DCM/MeOH (1:1)	P11788B	5.6	0.0	54.0	22.3	31.7	17.1	45.2	3.5
Asteraceae	***Brachylaena discolor***	Roots	DCM/MeOH (1:1)	P04199B	20.9	10.3	35.0	7.4	28.9	14.5	38.2	4.8
Asteraceae	***Brachylaena discolor***	leaves	DCM/MeOH (1:1)	P04321B	50.0	12.6	34.2	15.3	30.4	19.0	71.5	10.3
Asteraceae	***Brachylaena discolor***	Roots	DCM/MeOH (1:1)	P04459B	37.0	16.0	37.4	15.4	36.4	18.8	60.2	15.9

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Euphorbiaceae	***Bridelia micrantha***	leaves	DCM/MeOH (1:1)	P04722B	98.0	0.0	38.2	11.6	30.7	0.0	84.3	12.8
Euphorbiaceae	***Bridelia micrantha***	Stems	DCM/MeOH (1:1)	P04806B	58.4	24.3	37.5	13.7	54.0	20.0	92.5	23.3
Euphorbiaceae	***Bridelia micrantha***	Roots	DCM/MeOH (1:1)	P04815B	42.0	0.0	30.6	14.3	15.8	0.0	80.7	34.6
Rubiaceae	***Canthium mundianum***	Leaves	DCM/MeOH (1:1)	P00413B	12.8	0.0	48.9	20.2	30.7	23.0	24.1	0.0
Rubiaceae	***Canthium mundianum***	Leaves	MeOH	P00413C	10.1	0.0	45.0	16.3	30.8	28.3	22.6	0.0
Rubiaceae	***Canthium mundianum***	Roots	DCM	P00414A	23.1	0.0	49.9	22.3	65.7	22.4	87.1	15.5
Rubiaceae	***Canthium mundianum***	Roots	DCM/MeOH (1:1)	P00414B	14.5	0.0	49.9	18.5	31.8	21.4	29.6	0.0
Rubiaceae	***Canthium mundianum***	Roots	MeOH	P00414C	12.8	0.0	43.0	24.7	29.4	19.8	30.1	0.0
Rubiaceae	***Canthium mundianum***	Fruits	DCM/MeOH (1:1)	P00415B	10.6	0.0	46.7	20.3	20.5	19.0	26.3	0.0
Rubiaceae	***Canthium mundianum***	Fruits	MeOH	P00415C	0.7	0.0	38.1	9.7	0.0	0.0	25.2	0.1
Capparaceae	***Capparis tomentosa Lam.***	Roots	DCM/MeOH (1:1)	P06167B	31.2	30.2	30.5	13.1	35.1	23.8	76.3	26.4
		Fruits	DCM/MeOH (1:1)	P06168B	17.7	0.0	24.8	15.7	17.6	19.6	37.8	3.7
		Stems	DCM/MeOH (1:1)	P06169B	34.0	3.4	67.1	17.2	86.5	32.7	93.5	30.9
Asteraceae	***Cirsium vulgare***	Whole plant	DCM	P02112A	10.2	0.0	49.1	30.2	33.1	24.9	46.3	0.0
		Whole plant	DCM/MeOH (1:1)	P02112B	23.2	5.3	42.9	29.3	30.7	27.6	49.1	0.0
		Leaves	DCM/MeOH (1:1)	P06377B	11.3	1.4	42.2	10.2	39.7	18.4	81.0	20.6
		Roots	DCM/MeOH (1:1)	P06381B	28.7	1.8	8.3	5.3	38.2	16.7	51.9	11.8
Asteraceae	***Cirsium vulgare***	Stems	DCM/MeOH (1:1)	P06384B	5.9	0.0	46.8	20.6	35.3	15.2	70.6	16.5
rutaceae	***Clausena anisata***	leaves	DCM/MeOH (1:1)	P04413B	98.8	13.6	28.8	8.7	53.3	4.5	77.8	15.4

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
rutaceae	***Clausena anisata***	Roots	DCM/MeOH (1:1)	P04411B	82.5	6.3	25.0	7.1	71.4	1.4	100.0	51.5
Verbebaceae	***Clerodendrumglabrumglabrum***	Stems	DCM/MeOH (1:1)	P09466B	5.5	0.0	42.3	15.3	42.8	18.7	82.5	19.1
Verbebaceae	***Clerodendrumglabrumglabrum***	Roots	DCM/MeOH (1:1)	P09467B	19.7	0.0	50.0	17.7	69.8	31.5	58.6	4.5
Euphorbiaceae	***Clutiapulchellapulchella***	Twigs	DCM	P00020A	18.2	6.8	28.8	16.7	38.2	21.3	69.4	2.4
Euphorbiaceae	***Clutiapulchellapulchella***	Twigs	DCM/MeOH (1:1)	P00020B	20.0	10.8	37.6	23.1	33.3	20.9	38.0	2.7
Euphorbiaceae	***Clutiapulchellapulchella***	Twigs	MeOH	P00020C	18.3	9.9	38.1	16.5	31.9	19.3	36.0	0.0
Euphorbiaceae	***Clutiapulchellapulchella***	Leaves	DCM/MeOH (1:1)	P00476B	11.8	4.7	48.2	24.6	34.3	24.9	59.9	1.1
Euphorbiaceae	***Clutiapulchellapulchella***	Leaves	MeOH	P00476C	3.8	0.0	48.5	22.6	31.2	20.6	53.1	8.3
Euphorbiaceae	***Clutiapulchellapulchella***	Roots	DCM/MeOH (1:1)	P10738B	12.5	1.9	47.6	16.6	14.2	0.0	97.7	75.0
Euphorbiaceae	***Clutiapulchellapulchella***	Leaves & stems	DCM/MeOH (1:1)	P10739B	42.3	8.3	34.7	19.9	40.1	14.1	74.0	12.5
Euphorbiaceae	***Clutiapulchellapulchella***	Stems	DCM/MeOH (1:1)	P10740B	19.2	5.0	40.2	24.3	48.3	17.1	76.8	19.1
Euphorbiaceae	***Croton gratissimus***	Leaves	DCM/MeOH (1:1)	P07086B	40.1	3.2	38.0	15.6	96.4	26.1	71.8	27.1
Euphorbiaceae	***Croton gratissimus***	Stems	DCM/MeOH (1:1)	P07087B	38.9	6.4	25.3	12.7	60.6	0.0	83.2	42.9
Euphorbiaceae	***Croton gratissimus***	Bark	DCM/MeOH (1:1)	P07089B	100.0	1.9	13.3	16.0	67.2	29.6	79.6	31.0
Iridaceae	***Dietes iridioides***	Leaves	DCM/MeOH (1:1)	P08597B	98.8	0.0	43.5	22.9	30.4	18.1	69.4	8.4
Iridaceae	***Dietes iridioides***	Roots	DCM/MeOH (1:1)	P08598B	97.9	0.0	35.0	0.0	0.0	0.0	77.7	24.0
Iridaceae	***Dietes iridioides***	Flowers	DCM/MeOH (1:1)	P08599B	99.9	0.0	41.8	13.1	55.8	17.1	69.9	24.8
Iridaceae	***Dietes iridioides***	Fruits	DCM/MeOH (1:1)	P08600B	100.0	4.7	31.3	12.2	42.8	19.3	76.9	12.1
Apocynaceae	***Diplorhynchus condylocarpon***	Leaves	DCM	P00933A	20.6	0.0	49.0	26.5	80.4	28.2	78.2	9.9

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Apocynaceae	***Diplorhynchus condylocarpon***	Leaves	DCM/MeOH (1:1)	P00933B	6.0	0.0	46.0	16.1	35.5	25.2	37.9	0.0
Apocynaceae	***Diplorhynchus condylocarpon***	Roots	DCM	P00940A	12.7	0.0	38.9	18.2	57.3	27.2	43.8	0.0
Apocynaceae	***Diplorhynchus condylocarpon***	Roots	DCM/MeOH (1:1)	P00940B	18.6	0.0	58.7	13.9	35.3	26.5	61.7	0.0
Apocynaceae	***Diplorhynchus condylocarpon***	Twigs	DCM/MeOH (1:1)	P03406B	14.7	0.0	50.0	17.8	54.6	15.7	73.0	10.0
Apocynaceae	***Diplorhynchus condylocarpon***	Leaves & Twigs	DCM/MeOH (1:1)	P03407B	40.0	3.0	34.5	5.3	86.2	18.9	97.4	19.5
Apocynaceae	***Diplorhynchus condylocarpon***	Stems	DCM/MeOH (1:1)	P07108B	21.1	1.9	40.2	10.3	47.1	24.9	93.3	28.2
Euphorbiaceae	***Drypetes gerrardii***	Stems	DCM/MeOH (1:1)	P05778B	60.7	28.5	30.9	0.0	80.5	24.9	100.0	99.5
Euphorbiaceae	***Drypetes gerrardii***	Leaves	DCM/MeOH (1:1)	P05800B	96.5	37.3	32.0	6.9	28.1	23.6	69.9	21.6
Meliaceae	***Ekebergia capensis***	Leaves	DCM/MeOH (1:1)	P04616B	98.8	0.9	39.3	13.3	50.6	0.0	71.9	13.8
Meliaceae	***Ekebergia capensis***	Roots	DCM/MeOH (1:1)	P04929B	100.0	100.0	102.4	95.3	100.0	56.9	98.6	57.0
Euphorbiaceae	***Euphorbia clavarioides***	Leaves	DCM/MeOH (1:1)	P01559B	7.6	0.0	37.8	32.4	42.5	12.5	59.9	12.7
Euphorbiaceae	***Euphorbia clavarioides***	Roots	DCM/MeOH (1:1)	P01568B	17.2	0.0	46.8	25.5	45.9	17.4	63.9	5.4
Euphorbiaceae	***Euphorbia pulcherrima***	Leaves	DCM/MeOH (1:1)	P10453B	11.8	0.0	30.7	21.1	42.8	20.4	63.4	6.3
Euphorbiaceae	***Euphorbia pulcherrima***	Stems	DCM/MeOH (1:1)	P10454B	17.8	0.0	45.6	15.4	55.2	22.9	86.5	16.4
Euphorbiaceae	***Euphorbia pulcherrima***	Roots	DCM/MeOH (1:1)	P10455B	13.5	0.0	45.4	17.4	42.7	18.5	81.7	23.2
Asteraceae	***Felicia filifolia***	Leaves	DCM/MeOH (1:1)	P10676B	7.3	1.1	45.2	11.5	0.0	14.9	88.2	19.3
Asteraceae	***Helichrysum mundtii***	Stems	DCM	P00464A	19.3	0.0	79.0	34.9	64.7	31.0	84.3	20.7
Asteraceae	***Helichrysum mundtii***	Stems	DCM/MeOH (1:1)	P00464B	9.9	4.4	75.4	33.2	61.5	28.0	81.1	14.2
Asteraceae	***Helichrysum mundtii***	Stems	MeOH	P00464C	2.0	0.0	59.2	26.7	38.4	29.5	34.9	0.0

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Asteraceae	***Helichrysum mundtii***	Roots	DCM	P00466A	38.8	0.0	89.9	44.2	80.7	35.1	89.7	19.9
Asteraceae	***Helichrysum mundtii***	Roots	DCM/MeOH (1:1)	P00466B	15.2	0.7	73.7	32.8	47.8	27.4	71.5	3.2
Asteraceae	***Helichrysum mundtii***	Roots	MeOH	P00466C	17.3	0.0	58.9	28.0	36.9	23.9	55.2	0.0
Asteraceae	***Helichrysum mundtii***	Leaves	DCM/MeOH (1:1)	P00467B	7.7	0.0	52.2	19.0	32.3	21.2	46.5	0.3
Asteraceae	***Helichrysum mundtii***	Leaves	MeOH	P00467C	5.5	0.0	46.1	15.2	0.0	0.0	37.1	3.5
Asteraceae	***Helichrysum pedunculatum***	Whole plants	DCM/MeOH (1:1)	P08309B	9.2	0.0	40.0	8.1	36.3	31.8	61.9	24.0
Apiaceae	***Heteromorpha arborescens***	Roots	DCM/MeOH (1:1)	P12488B	17.3	0.0	40.2	26.8	38.0	32.0	66.2	27.7
Apiaceae	***Heteromorpha arborescens***	Bark	DCM/MeOH (1:1)	P12489B	19.0	0.0	33.4	14.9	44.8	28.2	84.0	26.5
Lamiaceae	***Hyptis pectinata (L.) Poit.***	Leaves,stems,fruit	DCM/MeOH (1:1)	P02459B	32.2	0.0	53.4	17.4	53.5	18.2	85.0	19.9
Proteaceae	***Leucospermum cuneiforme***	Leaves	DCM/MeOH (1:1)	P02599B	21.8	6.4	44.0	23.1	50.6	10.5	99.4	21.3
Proteaceae	***Leucospermum cuneiforme***	Roots	DCM/MeOH (1:1)	P02606B	18.5	3.3	49.0	19.7	11.1	3.5	41.2	9.9
Euphorbiaceae	***Macaranga capensis***	Leaves	DCM/MeOH (1:1)	P04676B	83.4	7.9	55.0	17.2	49.6	0.0	91.7	22.0
Sapotaceae	***Mimusops zeyheri* Sond.**	Leaves	DCM	P00488A	18.1	0.0	49.2	25.9	42.5	22.9	71.1	3.4
Sapotaceae	***Mimusops zeyheri* Sond.**	Leaves	MeOH	P00488C	99.3	0.0	50.8	24.2	28.5	23.3	30.1	0.0
Sapotaceae	***Mimusops zeyheri* Sond.**	Fruits	DCM	P00489A	18.6	0.0	48.0	27.0	40.6	26.8	53.2	0.0
Sapotaceae	***Mimusops zeyheri* Sond.**	Fruits	MeOH	P00489C	6.7	0.0	45.2	27.9	48.5	26.2	26.2	0.0
Sapotaceae	***Mimusops zeyheri* Sond.**	Roots	DCM	P00490A	10.3	0.0	46.7	22.1	45.9	27.9	83.4	9.3
Sapotaceae	***Mimusops zeyheri* Sond.**	Roots	MeOH	P00490C	9.4	0.0	41.6	23.3	35.0	22.9	27.3	0.0

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Sapotaceae	***Mimusops zeyheri* Sond.**	Stems	DCM	P00491A	12.8	0.0	40.9	29.0	56.0	27.2	88.7	14.8
Sapotaceae	***Mimusops zeyheri* Sond.**	Stems	MeOH	P00491C	17.6	0.0	48.1	27.8	27.7	21.9	23.8	0.0
Sapotaceae	***Mimusops zeyheri* Sond.**	Leaves & twigs	DCM	P02237A	34.0	0.0	48.0	25.3	61.6	27.9	98.5	16.6
Sapotaceae	***Mimusops zeyheri* Sond.**	Leaves & twigs	DCM/MeOH (1:1)	P02237B	99.5	0.0	42.5	24.9	27.4	23.0	26.4	0.0
Euphorbiaceae	***Monadenium lugardiae***	Whole plants	DCM/MeOH (1:1)	P02587B	97.8	2.3	40.6	15.8	69.5	16.1	97.7	27.7
Lamiaceae	***Ocimum gratissimum***	Leaves	DCM	P00766A	12.3	0.0	47.9	29.2	55.5	14.6	88.7	25.7
Lamiaceae	***Ocimum gratissimum***	Leaves	DCM/MeOH (1:1)	P00766B	0.3	0.0	43.2	21.3	0.0	0.0	51.9	21.0
Lamiaceae	***Ocimum gratissimum***	Roots	DCM/MeOH (1:1)	P00775B	6.7	4.8	45.6	11.8	30.5	11.6	22.0	10.8
Lamiaceae	***Ocimum gratissimum***	Stems	DCM	P00777A	26.5	3.7	39.0	22.9	52.9	17.9	90.5	29.2
Lamiaceae	***Ocimum gratissimum***	Stems	DCM/MeOH (1:1)	P00777B	0.5	0.0	44.3	22.0	33.6	11.5	28.4	0.0
Asteraceae	***Oedera genistifolia* (L.)**	Whole plant	DCM/MeOH (1:1)	P03206B	100.0	100.0	50.8	18.6	100.0	69.8	100.0	99.3
Asteraceae	***Othonna carnosa***	Stems	DCM/MeOH (1:1)	P02700B	100.0	2.3	43.9	17.1	29.0	3.8	98.5	37.8
Anacardiaceae	***Ozoroa sphaerocarpa***	Whole plant	DCM	P00437A	100.0	67.1	41.9	23.2	87.1	22.8	74.7	15.7
Anacardiaceae	***Ozoroa sphaerocarpa***	Whole plant	DCM/MeOH (1:1)	P00437B	100.0	0.0	51.1	24.0	82.7	26.1	81.5	18.1
Anacardiaceae	***Ozoroa sphaerocarpa***	Whole plant	MeOH	P00437C	99.6	0.0	40.2	10.9	33.3	24.1	39.1	0.0
Anacardiaceae	***Ozoroa sphaerocarpa***	Stems	DCM	P00439A	3.6	0.0	42.7	15.8	97.4	23.7	85.9	25.1
Anacardiaceae	***Ozoroa sphaerocarpa***	Stems	DCM/MeOH (1:1)	P00439B	99.1	0.0	40.3	29.5	39.9	23.3	54.9	0.0
Anacardiaceae	***Ozoroa sphaerocarpa***	Stems	MeOH	P00439C	99.6	0.0	35.1	21.9	30.1	25.3	31.2	1.4
Anacardiaceae	***Ozoroa sphaerocarpa***	Leaves	DCM/MeOH (1:1)	P00440B	100.0	0.0	34.2	24.3	43.7	22.6	47.7	0.0

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Anacardiaceae	***Ozoroa sphaerocarpa***	Leaves	DCM	P00440A	98.3	0.0	42.2	20.4	80.2	25.2	82.0	13.4
Anacardiaceae	***Ozoroa sphaerocarpa***	Leaves	MeOH	P00440C	100.0	0.0	42.3	26.7	32.0	20.8	22.8	0.0
Sapindaceae	***Pappea capensis***	Roots	DCM	P00397A	100.0	9.7	34.0	17.6	40.5	14.8	76.0	100.0
Sapindaceae	***Pappea capensis***	Roots	DCM/MeOH (1:1)	P00397B	0.1	0.0	40.0	14.7	0.0	0.0	37.4	4.5
Sapindaceae	***Pappea capensis***	Roots	MeOH	P00397C	19.5	0.0	46.0	26.6	30.7	25.3	36.7	1.1
Sapindaceae	***Pappea capensis***	Leaves	DCM/MeOH (1:1)	P10670B	99.7	0.0	17.6	15.5	15.5	0.0	79.2	35.9
Sapindaceae	***Pappea capensis***	Bark	DCM/MeOH (1:1)	P10671B	14.5	0.0	29.7	22.4	30.6	22.2	78.3	25.8
Sapindaceae	***Pappea capensis***	Roots	DCM/MeOH (1:1)	P10673B	15.2	0.0	29.5	25.7	44.5	25.4	95.9	54.1
Geraniaceae	***Pelargonium zonale***	Stems	DCM	P01688A	1.7	0.0	43.4	5.1	26.0	23.6	30.9	0.0
Geraniaceae	***Pelargonium zonale***	Stems	DCM/MeOH (1:1)	P01688B	99.4	0.0	40.0	21.8	28.7	24.1	33.5	0.0
Geraniaceae	***Pelargonium zonale***	Twigs	DCM/MeOH (1:1)	P03391B	15.7	4.1	44.4	19.9	26.9	17.2	52.1	5.1
Geraniaceae	***Pelargonium***	Leaves	DCM/MeOH (1:1)	P03392B	16.4	2.8	43.4	11.2	24.0	10.1	88.2	3.9
Fabaceae	***Peltophorum africanum***	Roots	DCM	P00394A	99.9	26.0	34.2	18.6	44.9	21.0	84.3	17.4
Fabaceae	***Peltophorum africanum***	Roots	DCM/MeOH (1:1)	P00394B	100.0	8.2	34.9	18.0	26.2	20.6	54.0	2.0
Fabaceae	***Peltophorum africanum***	Roots	MeOH	P00394C	100.0	6.1	32.1	12.2	23.9	21.7	34.8	0.0
Fabaceae	***Peltophorum africanum***	Stems	DCM	P00401A	99.6	49.3	60.7	29.0	31.5	22.4	90.2	31.6
Fabaceae	***Peltophorum africanum***	Stems	DCM/MeOH (1:1)	P00401B	100.0	10.6	52.4	25.2	28.8	21.6	63.8	11.4
Fabaceae	***Peltophorum africanum***	Stems	MeOH	P00401C	100.0	11.6	51.6	15.2	29.8	22.0	66.5	9.2
Fabaceae	***Peltophorum africanum***	Leaves & twigs	DCM	P02244A	100.0	0.0	56.5	23.9	78.3	28.5	98.8	29.6

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Fabaceae	***Peltophorum africanum***	Leaves & twigs	DCM/MeOH (1:1)	P02244B	57.6	0.0	40.6	22.1	28.2	22.8	49.7	3.7
Fabaceae	***Peltophorum africanum***	leaves & twigs	MeOH	P02244C	95.4	0.0	40.5	6.7	0.0	0.0	30.3	0.0
Asteraceae	***Phymaspermum acerosum***	Whole plant	DCM/MeOH (1:1)	P03661B	100.0	13.1	44.5	13.1	67.7	17.8	98.6	37.8
Lamiaceae	***Plectranthus laxiflorus.***	Whole plants	DCM/MeOH (1:1)	P12870B	92.1	9.9	40.0	14.4	51.5	29.0	59.9	14.6
Polygalaceae	***Polygala fruticosa***	Leaves	DCM/MeOH (1:1)	P03756B	37.3	14.4	68.9	34.7	65.6	0.0	98.6	35.6
Polygalaceae	***Polygala fruticosa***	Twigs	DCM/MeOH (1:1)	P03757B	24.7	11.7	46.8	17.8	35.3	0.0	82.5	23.2
Fabaceae	***Psoralea pinnata***	Leaves	DCM	P01617A	98.7	100.0	36.6	30.0	91.9	26.9	98.8	19.0
Fabaceae	***Psoralea pinnata***	Leaves	DCM/MeOH (1:1)	P01617B	99.7	99.2	32.1	19.8	73.3	22.7	83.9	0.6
Fabaceae	***Psoralea pinnata***	Stems	DCM	P01676A	7.5	0.0	52.8	20.5	32.4	15.9	52.4	1.9
Fabaceae	***Psoralea pinnata***	Stems	DCM/MeOH (1:1)	P01676B	4.3	0.0	47.3	16.4	0.0	0.0	38.5	7.1
Fabaceae	***Psoralea pinnata***	Roots	DCM	P01679A	8.6	0.0	44.7	24.1	35.5	23.2	41.0	0.0
Fabaceae	***Psoralea pinnata***	Roots	MeOH	P01679C/B	8.2	0.0	44.9	12.4	24.5	21.2	39.3	0.0
Rubiaceae	***Psychotria capensis***	Roots	DCM/MeOH (1:1)	P09181B	17.4	0.0	31.1	13.0	39.0	21.4	28.9	5.5
Rubiaceae	***Psychotria capensis***	Leaves	DCM/MeOH (1:1)	P09182B	18.4	0.0	30.6	14.8	37.4	20.4	36.7	10.3
Rubiaceae	***Psychotria capensis***	Stems	DCM/MeOH (1:1)	P09183B	99.9	0.0	36.7	3.1	39.4	19.1	30.1	0.0
Lamiaceae	***Rabdosiella calycina***	Stems	DCM	P00451A	98.4	0.0	45.3	8.6	103.4	40.6	77.5	11.3
Lamiaceae	***Rabdosiella calycina***	Stems	DCM/MeOH (1:1)	P00451B	38.1	0.0	48.8	23.2	14.1	0.0	49.2	2.8
Lamiaceae	***Rabdosiella calycina***	Stems	MeOH	P00451C	99.9	0.0	44.4	18.3	59.7	24.6	51.2	9.6
Lamiaceae	***Rabdosiella calycina***	Leaves	DCM/MeOH (1:1)	P00453B	5.4	0.0	52.0	16.0	33.3	21.1	33.0	0.0

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Lamiaceae	***Rabdosiella calycina***	Leaves	MeOH	P00453C	0.0	0.0	44.4	26.5	32.5	21.9	27.9	0.0
Lamiaceae	***Rabdosiella calycina***	Whole plant	DCM/MeOH (1:1)	P08318B	99.6	0.0	44.1	12.1	80.4	22.6	76.9	20.6
Myrsinaceae	***Rapanea melanophloeos***	Leaves	DCM/MeOH (1:1)	P08222B	19.9	34.7	37.3	14.8	60.6	30.8	44.7	5.1
Myrsinaceae	***Rapanea melanophloeos***	Twigs	DCM/MeOH (1:1)	P08223B	95.8	0.0	41.3	15.5	100.0	28.5	43.1	10.8
Myrsinaceae	***Rapanea melanophloeos***	Stems	DCM/MeOH (1:1)	P09405B	33.3	0.0	32.1	8.7	38.7	17.8	16.6	0.6
Myrsinaceae	***Rapanea melanophloeos***	Roots	DCM/MeOH (1:1)	P09406B	99.0	0.0	38.3	18.6	67.0	20.6	17.8	13.0
Vitaceae	***Rhoicissus tridentata***	Leaves	DCM/MeOH (1:1)	P09322B	82.2	0.0	46.0	16.8	39.5	18.5	37.0	4.1
Vitaceae	***Rhoicissus tridentata***	Fruits	DCM/MeOH (1:1)	P09323B	99.7	0.0	41.1	17.6	47.0	25.7	54.3	11.7
Vitaceae	***Rhoicissus tridentata***	Roots	DCM/MeOH (1:1)	P09324B	0.0	0.0	46.7	24.6	38.5	20.8	30.4	9.9
Vitaceae	***Rhoicissus tridentata***	Bark	DCM/MeOH (1:1)	P09325B	97.9	0.0	44.4	19.8	29.9	17.7	32.1	1.0
Vitaceae	***Rhoicissus tridentata***	Stems	DCM/MeOH (1:1)	P09326B	13.5	0.0	45.2	22.1	26.5	14.8	50.9	2.3
Apocynaceae	***Sarcostemma viminale***	Leaves	MeOH	P00363C	18.1	2.2	33.4	15.3	36.4	20.4	49.6	7.4
Apocynaceae	***Sarcostemma viminale***	Stems	DCM	P01381A	12.1	0.0	40.1	27.4	38.4	23.8	70.0	2.7
Apocynaceae	***Sarcostemma viminale***	Whole plant	DCM/MeOH (1:1)	P02503B	33.8	7.1	46.4	21.7	18.3	4.3	74.9	12.9
Apocynaceae	***Sarcostemma viminale***	Roots	DCM/MeOH (1:1)	P06980B	99.5	7.0	46.3	20.0	57.6	24.3	99.3	31.8
Asclepiadaceae	***Sarcostemma viminale***	Stems	DCM/MeOH (1:1)	P01381B	6.5	0.0	46.0	17.3	29.7	22.2	35.5	0.0
Asteraceae	***Schkuhria pinnata***	whole plants	DCM/MeOH (1:1)	P03491B	99.4	97.1	67.4	16.9	69.7	0.0	100.0	73.8
Fabaceae	***Senna septemtrionalis***	Roots	DCM/MeOH (1:1)	P06145B	43.0	5.9	33.8	17.2	24.9	22.0	45.6	14.4
Fabaceae	***Senna septemtrionalis***	Stems	DCM/MeOH (1:1)	P06146B	98.5	11.4	37.5	10.7	23.8	15.0	45.4	15.3

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Fabaceae	***Senna septemtrionalis***	Leaves	DCM/MeOH (1:1)	P06147B	39.7	9.7	40.2	18.7	33.9	13.4	83.7	18.5
Fabaceae	***Senna septemtrionalis***	Seeds	DCM/MeOH (1:1)	P06148B	0.0	0.0	34.7	12.7	0.5	0.0	33.6	11.4
Apiaceae	***Steganotaenia araliacea***	Roots	DCM	P00758A	20.6	0.0	52.5	44.4	35.1	24.3	64.9	11.0
Malvaceae	***Sterculia rogersii*.**	Leaves	DCM/MeOH (1:1)	P03870B	100.0	13.0	32.8	8.6	44.7	18.4	61.6	18.6
Malvaceae	***Sterculia rogersii***	Roots	DCM/MeOH (1:1)	P03872B	14.1	5.9	35.8	13.8	39.9	14.7	42.4	15.6
Malvaceae	***Sterculia rogersii***	Bark	DCM/MeOH (1:1)	P03873B	32.9	33.7	25.1	12.5	62.0	18.4	91.9	30.2
Rutaceae	***Toddalia asiatica***	Roots	DCM/MeOH (1:1)	P00599B	98.1	0.0	45.4	17.4	36.0	20.8	94.2	25.6
Rutaceae	***Toddalia asiatica***	Leaves & twigs	DCM/MeOH (1:1)	P02460B	16.7	0.0	40.3	14.2	53.5	20.2	68.5	14.3
Ulmaceae	***Trema orientalis***	Leaves	DCM/MeOH (1:1)	P11527B	98.3	0.0	82.3	10.9	100.0	27.6	94.5	24.3
Ulmaceae	***Trema orientalis (L.) Blume***	Flowers	DCM/MeOH (1:1)	P11528B	3.6	0.0	36.2	14.3	41.7	23.0	85.0	21.2
Ulmaceae	***Trema orientalis (L.) Blume***	Roots	DCM/MeOH (1:1)	P11529B	14.4	2.2	49.7	27.3	34.0	17.1	74.5	13.2
Ulmaceae	***Trema orientalis (L.) Blume***	Stems	DCM/MeOH (1:1)	P11530B	11.7	3.1	46.9	18.3	0.0	19.2	88.8	27.1
Meliaceae	***Turraea floribunda*** **Hochst.**	Bark	DCM/MeOH (1:1)	P05214B	93.5	12.8	28.3	15.6	28.4	20.4	89.9	39.9
Meliaceae	***Turraea floribunda*** **Hochst.**	leaves	DCM/MeOH (1:1)	P05294B	100.0	14.2	38.1	22.3	39.7	22.2	78.3	27.4
Meliaceae	***Turraea floribunda*** **Hochst.**	Roots	DCM/MeOH (1:1)	P05329B	64.4	10.0	18.3	18.8	53.6	21.3	98.9	58.6
Rubiaceae	***Vangueria infausta***	Fruits	DCM	P00008A	29.5	18.9	31.2	7.4	0.0	0.0	35.7	3.1
Rubiaceae	***Vangueria infausta***	Fruits	DCM/MeOH (1:1)	P00008B	21.6	13.3	43.1	14.1	23.1	20.0	33.6	2.6
Rubiaceae	***Vangueria infausta***	Fruits	MeOH	P00008C	17.3	7.6	45.0	16.0	31.5	21.4	30.4	0.0
Rubiaceae	***Vangueria infausta***	Roots	DCM/MeOH (1:1)	P09344B	17.4	5.2	37.4	14.6	16.8	0.0	44.6	14.1

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
Rubiaceae	***Vangueria infausta***	Stems	DCM/MeOH (1:1)	P09345B	22.2	10.2	38.1	14.9	38.1	17.3	43.9	11.6
Rubiaceae	***Vangueria infausta***	Leaves	DCM/MeOH (1:1)	P09346B	99.2	0.0	36.9	10.5	45.5	19.8	52.9	15.2
Asteraceae	***Vernonia mespilifolia* Less**	Leaves	DCM/MeOH (1:1)	P11137B	100.0	100.0	90.1	34.5	100.0	29.5	96.6	32.5
Asteraceae	***Vernonia mespilifolia* Less**	Stems	DCM/MeOH (1:1)	P11138B	95.1	0.0	35.1	25.9	84.5	29.8	55.8	8.4
Asteraceae	***Vernonia mespilifolia* Less**	Roots	DCM/MeOH (1:1)	P11139B	16.8	0.0	38.9	21.5	36.4	26.1	48.4	9.6
Asteraceae	***Vernonia myriantha***	Roots	DCM	P00170A	7.7	0.0	37.4	14.5	0.0	0.0	36.9	15.8
Asteraceae	***Vernonia myriantha***	Roots	DCM/MeOH (1:1)	P00170B	13.6	4.3	46.0	24.6	32.5	17.0	37.0	12.2
Asteraceae	***Vernonia myriantha***	Leaves	DCM	P00171A	8.6	0.0	36.5	19.3	39.0	16.6	59.5	7.5
Asteraceae	***Vernonia myriantha***	Leaves	DCM/MeOH (1:1)	P00171B	1.5	0.0	30.5	22.1	36.0	21.5	29.2	1.9
Asteraceae	***Vernonia myriantha***	Leaves	MeOH	P00171C	0.2	0.0	39.1	23.5	36.4	26.5	26.3	6.2
Asteraceae	***Vernonia myriantha***	Stems	DCM	P00172A	5.3	0.0	40.0	17.0	37.4	25.6	32.6	14.9
Asteraceae	***Vernonia myriantha***	Stems	MeOH	P00172C	0.0	0.0	43.2	14.8	32.6	21.1	17.0	0.0
Asteraceae	***Vernonia myriantha***	Seeds	DCM	P00173A	0.0	0.0	39.6	20.0	35.7	19.0	32.9	0.4
Asteraceae	***Vernonia myriantha***	Seeds	DCM/MeOH (1:1)	P00173B	0.0	0.0	35.8	18.1	38.0	22.1	36.9	0.1
Olacaceae	***Ximenia caffra***	Leaves	DCM/MeOH (1:1)	P03508B	25.3	15.2	42.7	17.3	33.9	26.7	51.9	0.0
Olacaceae	***Ximenia***	Twigs	DCM/MeOH (1:1)	P03509B	40.7	0.0	32.7	10.8	0.0	0.0	50.1	9.2
rutaceae	***Zanthoxylum capense***	Leaves	DCM/MeOH (1:1)	P07947B	34.0	0.0	39.5	16.6	46.1	30.1	99.7	38.8
rutaceae	***Zanthoxylum capense***	Stems	DCM/MeOH (1:1)	P07948B	27.5	5.7	39.0	18.8	51.7	27.0	95.4	40.0
rutaceae	***Zanthoxylum capense***	Roots	DCM/MeOH (1:1)	P07949B	11.6	34.3	44.2	7.2	47.5	29.3	86.1	32.5

					Percentage (%) growth inhibition							
					T. b. rhodesiense		*T cruzi*		*L. donovani*		*P. falciparum*	
Family	**Species**	**Plant part**	**Extract type**	**Extract no.**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**	**10 µg/ml**	**2 µg/ml**
rutaceae	***Zanthoxylum capense***	Fruits	DCM/MeOH (1:1)	P07950B	7.2	0.0	37.1	19.8	36.2	27.9	70.0	14.3
rutaceae	***Zanthoxylum davyi***	Leaves	DCM/MeOH (1:1)	P10430B	49.3	2.2	35.7	12.7	88.9	30.3	82.2	25.4
Rutaceae	***Zanthoxylum davyi***	Fruits	DCM/MeOH (1:1)	P10431B	5.2	0.0	32.0	7.9	30.4	26.1	44.3	8.5
Rutaceae	***Zanthoxylum davyi***	stems	DCM/MeOH (1:1)	P10432B	23.7	14.8	39.0	18.4	34.3	16.9	84.0	30.1

Table 4.2 The IC_{50} values (µg/mL), selectivity index and cytotoxicity of 18 South African plant species against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, *Plasmodium falciparum* and myoblast L6 cells. The positive controls melarsoprol[a], benznidazole[b], miltefosine[c], chloroquine[d] and podophyllotoxin[e] were tested likewise. Tests were done twice in duplicate.

				T. b. rhodesiense		***T. cruzi***		***L. donovani***		***P. falciparum* (NF-54 strain)**		**Cytotoxicity (L6-cells)**
Family	**Plant species**	**Plant part**	**Extract type**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**
Sapindaceae	*Pappea capensis*	Roots	DCM	15.40±1.84	2.43					10.10±2.79	3.70	37.40±22.42
Anacardiaceae	*Ozoroa sphaerocarpa*	Whole plant	DCM	10.90±7.09	4.76			5.78±0.25	8.98	12.90±7.42	4.02	51.90±3.75
Fabaceae	*Acacia erioloba*	Roots	DCM/MeOH (1:1)			18.50±11.29	3.01			10.70±2.74	5.21	55.70±7.92
Fabaceae	*Psoralea pinnata*	Leaves	DCM	0.15±0.02	97.32			5.08±0.11	2.85	8.46±5.03	1.71	14.50±2.83
Fabaceae	*Psoralea pinnata*	Leaves	DCM/MeOH (1:1)	0.31±0.02	139.61			6.73±0.16	6.39	9.18±1.61	4.68	43.00±14.64
Asteraceae	*Oedera genistifolia*	Whole plant	DCM/MeOH (1:1)	4.38±1.87	11.16	31.40±12.10	1.56	1.71±0.02	28.60	2.88±1.37	16.98	48.90±9.55
Fabaceae	*Abrus precatorius africanus*	Whole plant	DCM/MeOH (1:1)	1.04±0.45	48.17			8.84±0.11	5.67	3.39±0.07	14.78	50.10±2.05
Euphorbiaceae	*Clutia pulchella pulchella*	Roots	DCM/MeOH (1:1)							3.19±0.94	28.21	90.00±14.21
Euphorbiaceae	*Clutia pulchella pulchella*	Leaves & stems	DCM/MeOH (1:1)							14.10±5.73	3.74	52.70±1.20
Asteraceae	*Schkuhria pinnata*	Whole plant	DCM/MeOH (1:1)	2.04±0.02	8.63	17.90±10.02	0.98	10.80±4.05	1.63	2.19±0.48	8.04	17.60±0.99
Rutaceae	*Clausena anisata anisata*	Roots	DCM/MeOH (1:1)	7.19±0.95	3.59			11.50±5.21	2.24	3.61±1.82	7.15	25.80±5.73
Meliaceae	*Ekebergia capensis*	Roots	DCM/MeOH (1:1)	1.36±0.85	24.26	17.40±2.78	1.90	6.42±2.59	5.14	6.81±3.92	4.85	33.00±23.41

				T. b. rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum* (NF-54 strain)		Cytotoxicity (L6-cells)
Family	**Plant species**	**Plant part**	**Extract type**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**
Meliaceae	*Turraea floribunda*	Bark	DCM/MeOH (1:1)	24.40±12.16	2.28					4.52±0.53	12.32	55.70±16.26
Meliaceae	*Turraea floribunda*	Leaves	DCM/MeOH (1:1)	17.10±1.06	2.82					12.70±5.24	3.80	48.30±6.58
Meliaceae	*Turraea floribunda*	Roots	DCM/MeOH (1:1)	22.40±6.58	2.36			13.10±4.17	4.03	5.56±2.91	9.50	52.80±7.00
Meliaceae	*Drypetes gerrardii*	Stems	DCM/MeOH (1:1)	8.39±5.54	6.05			7.31±0.85	6.95	0.50±0.23	101.20	50.80±1.77
Meliaceae	*Drypetes gerrardii*	Leaves	DCM/MeOH (1:1)	12.10±0.28	4.49					21.60±0.64	2.51	54.30±2.90
Asteraceae	*Helichrysum pedunculatum*	Whole plant	DCM/MeOH (1:1)					13.50±2.90	4.29	6.46±3.44	8.96	57.90±11.17
Sapindaceae	*Pappea capensis*	Roots	DCM/MeOH (1:1)							5.30±1.75	16.00	84.80±21.57
Sapindaceae	*Pappea capensis*	Leaves	DCM/MeOH (1:1)	14.10±4.24	5.43					9.67±0.38	7.91	76.50±33.16
Asteraceae	*Vernonia mespilifolia Less.*	Leaves	DCM/MeOH (1:1)	1.01±0.31	14.65	8.76±12.01	1.69	2.48±0.33	5.97	5.09±1.86	2.91	14.80±3.25
	Standard drug			0.002±0.00[a]		0.355±0.09[b]		0.179±0.02[c]		0.003±0.00[d]		0.008±0.00[e]