Valorization of Angolan traditional herbal preparations used against trypanosomiasis

Inauguraldissertation
zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

von

Nina Vahekeni
aus Langnau i.E. (BE)

Basel, 2024

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

Auf Antrag von

Fakultätsverantwortlicher und Dissertationsleiter:	Prof. Dr. Pascal Mäser
Korreferent/in:	Prof. Dr. Philippe Christen

Basel, den 17. Dezember 2019

Prof. Dr. Marcel Mayor

Table of content

Acknowledgement

First of all, I would like to thank sincerely Pascal Mäser, my thesis Director, for having me given the opportunity to carry out this own elaborated research project. His solicitude and open-minded personality allowed me to use my creativity without any judgement. His enthusiastic support gave me the necessary courage to finalize this work.

Many thanks are also due to Peter van Eeuwijk, my supervisor in medical anthropology. His constant availability, his long experience from field studies, his sense of rigor and detail, are as many qualities and competences that allowed me to broaden my knowledge and my sense of analysis.

A very personal thank goes to Jacques Falquet, whose constant encouragement and experienced advises guided me all along the way of the work. His expertise in the ethnobotanical and ethnopharmacological field is a constant source of inspiration for me.

I would like to also thank Evelyn Wolfram, who very generously opened her lab to me to produce all the plant extracts. Her enthusiasm, spontaneity, and creativity are all human skills that lighted up my spirit. Her broad interest in natural compound chemistry is an example to me.

Many thanks to Marcel Kaiser, for his scientific expertise and advises.

Special thanks go to my PhD colleague from Geneva, Théo Brillatz, with whom a friendly and fruitful collaboration has been established. Thank you to Marjan Rahmati for the good collaboration, Laurence Marcourt for her crucial work in the interpretation of NMR spectra.

A particular thank goes to Monica Cal, Sonja Keller-Maerki und Romina Rocchetti for their helpful and expert technical assistance, for all the bioassay performed for me during my absence from Basel.

Many thanks to my colleagues Natalie Wiedemar, Anna Fesser, for our stimulating scientific exchange as well as friendship.

Many thanks are also due to Dr. João Pedro Guilherme for his advices, experience and determination which facilitated the implementation of the field work in Angola.

.

Thank the former Director of the ICCT, Prof. Dr. Josenando Théophile for giving the assurance of the need of this study, Dr. Amadeu and Dr. Paulo Makana for their support and Sr. Mafuta Junior and Sr. Felix Makiadi Donzoan for their constant disposability.

Many thanks are also due to the Coordinator of the CATEMATA, Sr. Kitoko Mayavângwa called *Papa Kitoko*, for helping me building trust with the traditional practitioners. I give thanks to

the former Director of the FOMETRA in Uíge, Sr. António Kiakubua for his role as “gate opener” and his long-lasting support.

A particular thank goes to PD Dr. Christian Schindler of the Swiss TPH who provided statistical guidance, to Ndombe Wandombe Filipe of ICCT who draw the maps, to Aristides of the CNIC who drove us safely into remote areas, to Dr. Amândio Luis Gomes from the UAN who helped us in the botanical identification of the plants’ species and to Sr Mambote from Uíge for his most valuable translation competence.

I would also like to acknowledge all persons, administrative personnel, students who shared a piece of this experience and contribute to its fulfillment.

Special thanks go to former Minister of Sciences and Technology, His Excellency Prof. Dr. Maria Cândida Texeira who made the implementation of this study work in Angola possible.

To my friends Marlène, Agnès, Mimi, Daniel, Salomon for their friendship and constant support.

To my brothers and sisters, who were always there to support me and find the right word.

I wish to express my sincere gratitude to my parents, who supported me in any way, and comforted me in my doubts.

Above all, to my dearest husband, who believed in this project, from the very beginning, and trusted me. Thank you for always being there when I need you. You are my light in my darkness.

To my two lovely sunshine, I am grateful for the joy and enthusiasm they bring into my life.

I apologize to everybody not mentioned here personally but involved in the success of this these. Many thanks to all of them.

Abbreviations

ABS	Access and Benefit Sharing
ATM	African Traditional Medicine
CATEMETA	Professional Chamber of Traditional, Alternative and non-Conventional Practitioners in Angola
CNIC	National Center of Scientific Investigation
CONMENTA	National Council of Traditional Natural Medicine in Angola
FM	Folk medicine
FOMETRA	Fórum da Medicina Tradicional em Angola
g-HAT	Gambiense trypanosomiasis
HAT	Human African Trypanosomiasis
ICCT	Instituto de Combate e Controlo das Triponossomíases
ISPSO	Swiss Institute of Pharmaceutical Sciences of Western Switzerland
INBAC	National Institute for Biodiversity and Conservation Areas
MAT	Mutually Agreed Terms
MOU	Memorandum of Understanding
NTD	Neglected Tropical Disease
OMS	Organização Mundial da Saúde
PCU	Parasite Chemotherapy Unit
Swiss TPH	Swiss Tropical and Public Health Institute
THA	Tripanossomíase Humana Africana
TM	Traditional Medicine
UAN	Universidade Agostinho Neto
WHO	World Health Organization
ZHAW	Zürcher Hochschule für Angewandten Wissenschaften

Summary

In Angola, Human African Trypanosomiasis is caused by the protozoal subspecies *Trypanosoma brucei gambiense* and is transmitted by tsetse flies (*Glossina* spp.). It is endemic in seven provinces of the country, and an estimated 5.8 million people are at risk. Despite availability of an effective standard treatment and control activities, a certain number of cases are undetected and untreated, these are mainly found among hard-to reach communities, much exposed to the disease with difficult access to healthcare. In such context, the investigation of herbal remedies as a natural affordable and accessible resource is of high relevance. There is a lack of information on Angolan medicinal plants currently used against trypanosomiasis. In the presented PhD study, I have investigated the actual use of traditional herbal remedies in the management of this disease. By investigation of the access to and the use of the traditional remedies, I aimed to fill up this gap by delivering useful information regarding the usage of the medicinal plants and the antitrypanosomal potential of the reported traditional recipes.

First, I implemented and conducted an exploratory study in four northern endemic provinces of Angola. The ethnomedical and ethnobotanical analysis revealed that among the infected persons, 40% turn to folk medicine before consulting a medical doctor. Moreover, 30 plant species were mentioned in the management of the disease, of which *Crossopteryx febrifuga* was the most cited plant. In addition, the study highlighted the use of plants with potential toxicity risk, as for example an herbal preparation containing the roots of *Aristolochia gigantea.*
I further selected 9 species (of 30) for *in vitro* antitrypanosomal screening, according to four selection criteria: (1) the Use Report, (2) the correlation between traditional reported preparation and clinical data, (3) the quality of the narrative content, and (4) the novelty of the plant.

I pursued with the bioguided-activity screening of the 9 plant species, out of which 122 plant extracts were produced. Two crude extracts, the 80% ethanolic extract of *Brasenia schreberi* (leave) and the dichloromethane extract of *Nymphaea lotus* (leaf and petiole) displayed IC_{50} values < 10 µg/ml against *Trypanosoma brucei rhodesiense*, and were retained for bioguided-fractionation and isolation of active constituents. 7 active phenols for *B. schreberi* , namely gallic acid **(1)**, methyl gallate **(2)**, tetragalloylglucose **(3)**, ethyl gallate **(4)**, 1,2,3,4,6 pentagallyl-β-glucopyranoside **(5)**, gossypetin-7-O-β-glucopyranoside **(6)**, hypolaetin-7-O-glucoside **(7)**, and 1 active compound for *N. lotus*, a resorcinol alkyl **(8)**. Compounds **(2-3**, **5-6)** were reported for the first time in the genus Brasenia and the presence of compound **(8)** was so far not described in the *Nymphaeacea.* The antitrypanosomal potential of the traditional preparation made of these two aquatic plants was assessed. We could evidence the presence of 3 active constituents in both decoctions: gallic acid, methyl gallate, and 1,2,3,4,6-Pentylgalloyl-β-glucopyranoside.

Taken together, this work provided primary evidence for the rational use of a traditionally used preparation made of *Brasenia schreberi* and *Nymphaea lotus*. As so, these results contributed to the scientific validation of an herbal remedy used in the management of sleeping sickness in Angola.

1 INTRODUCTION

1.1 Human African Trypanosomiasis and the situation in Angola

Human African trypanosomiasis is a Neglected Tropical Disease (NTD) that occurs only in Sub-Saharan Africa. African trypanosomiases are vector-borne diseases caused by protozoan parasites of the genus Trypanosoma. The species Trypanosoma brucei [T. b.] is responsible for African trypanosomiasis or sleeping sickness [1]. T. b. brucei is one of the etiologic agents of Animal African Trypanosomiasis (AAT), also called "nagana". T. b. brucei is not infective to humans. T. b. gambiense together with T. b. rhodesiense are responsible for Human African Trypanosomiasis (HAT). T. b. gambiense is endemic in West and Central Africa, including Angola. T. b. rhodesiense is restricted to East and Southeast Africa. These ranges do not overlap, although in Uganda both subspecies are co-endemic, with T. b. gambiense found near the northern border and T. b. rhodesiense in the central and southern regions[1].

Trypanosomes are transmitted from one mammalian host to another by blood-feeding tsetse flies (Glossina spp.). Once a tsetse fly has taken a blood meal on an infected person, the trypanosomes enter its digestive tract [2]. They undergo several differentiation steps and end up in the salivary glands of the insect for maturation. The parasite is transmitted when the fly bites another mammalian host. Thus, during his life cycle, T. brucei alternates between a mammal and an insect host (tsetse fly); it always remains extracellular.

Gambiense trypanosomiasis (g-HAT) is a chronifying disease that evolves in two phases. Stage 1 is the haemolymphatic stage, whose leading signs are nonspecific as for example chronic and intermittent fever, headache, pruritus and lymphadenopathy. Stage 1 can even remain asymptomatic [1]. As the symptoms are unspecific, the patients often do not feel the need to go for a health check. Stage 2 is the meningoencephalitic stage that is characterized by the invasion of the nervous system by trypanosomes and marked by sleep disturbances and neuropsychiatric disorders. The specificity and severity of the symptoms in stage 2 push patients to seek treatment. The disease leads to coma and death if left untreated. The estimated average duration of such infection is around three years [3]. Rhodesiense trypanosomiasis (r-HAT) is usually acute, and if not treated, death occurs within weeks or months [4].

To date, the recommended treatment for g-HAT relied on four medicines [5]: pentamidine, melarsoprol, nifurtimox, and eflornithine. Concerning (r-HAT), three medicines are recommended: pentamidine, suramin and melarsoprol. These reference drugs are donated by the manufacturers and distributed by WHO free of charge to guarantee that any patient worldwide has access to the most effective available treatment.
The choice of treatment depends on the sub-species of trypanosome and on the stage of the disease (Table 1). Until now, the first-line treatment for g-HAT was pentamidine for first-stage disease and nifurtimox-eflornithine combination therapy (NECT) for second-stage disease. Alternatively, eflornithine and melarsoprol were used as monotherapies in rare cases.

[1] https://www.cdc.gov/dpdx/trypanosomiasisafrican/index.html

Stage of disease	*g-HAT (T.b.gambiense)*	*r-HAT (T.b.rhodesiense)*
First stage	Pentamidine	Suramin
Second stage	NECT	Melarsoprol

Table 1-1: First-line treatment recommended by the WHO guideline.
NECT: nifurtimox- eflornithine combination therapy

Fexinidazole, a new medicine that received regulatory approval, has been introduced as the first-line treatment for g-HAT[6]. Fexinidazole is the first-ever oral treatment for all stages of g-HAT. The main advantages of this new drug are its use for both stages of the disease, the superfluity of doing the lumbar punctures for staging the disease, and its oral intake that does not require systematic hospitalization, which is of crucial importance for poor people leaving in remote areas. Trypanosomiasis mainly affects remote rural communities, where health infrastructure is basic and its access complicated[7]. This easy-to-use drug will facilitate the treatment and will increase adherence for people living at a critical distance from the health centers, thus increasing the coverage. When taken correctly for 10 days, fexinidazole presents equivalent efficacy to pentamidine in first-stage HAT and to NECT in second-stage HAT. However, its efficacy is inferior to NECT in the severe second-stage [8]. In Angola, while pentamidine is still prescribed for the first stage of the disease, the NECT therapy is used in replacement of melarsoprol.

An important measure for the control of the disease, introduced in the 1920s in Cameroon for the first time, is the use of mobile teams [9]. This method of systematic case detection and treatment enables to detect and treat patients before they reach stage 2, and to limit and possibly eliminate the parasite reservoir. Thus, the prevalence level of sleeping sickness can be drastically lowered. In Angola, active case detection method is run several times a year in specific foci, according to the number of reported new cases (see Fig. 1-1). However, if control measures are relaxed, for example in the context of conflict or socio-political instability, or economic crisis (as experienced from 2014 to 2016 due to slumps in oil prices), HAT can upsurge [10]. As example, during the civil war period affecting Angola from 1975 to 2002, the number of functional mobile teams for control activities were limited and the follow-up of infected persons was significantly reduced. Nowadays, as the epidemic faded away and the prevalence of the disease has been drastically reduced, the attendance to the case-finding campaigns of the local communities living in endemic areas is no longer of great priority[2].

[2] Personal communication from a member of the medical team of the Institute of Combate and Control of Trypanosomiasis (ICCT).

Figure 1-1: Control measures implemented in Angola. A: trucks of the ICCT for case-finding campaigns in rural areas**. B:** Case-detection activity in village. **C:** tsetse fly trap hanged out in a foci area of northern province Uíge.

The disease is controlled by a combination of approaches, including treatment of patients, active case finding, and vector control [11]. From the recent history, one can say that trypanosomiasis can be controlled probably not eradicated, due to fluctuations in socio economic situation and poverty of the affected population.

In 2017, 1442 cases were reported to the WHO. Progress in the Democratic Republic of Congo, which is the most affected country (660 reported new cases in 2018), resulted in drastic reduction in the number of cases[3].
The total population of Angola is estimated to 24 million [12], of whom an estimated 5.8 million people are at risk [13]. Sleeping sickness is found in the northwestern part of Angola. Of 18 provinces, seven are endemic: Luanda, Zaire, Uíge, Bengo, Kwanza Norte, Kwanza Sul and Malange [14] (Fig.1-2). Foci are well known with a low prevalence, and in general with a decreasing trend. The incidence reduced significantly throughout the last decade passing from 1105 new cases in 2007 to 79 cases in 2018 (Fig. 1-3). These numbers are low in comparison to the incidence rate during the civil war period (extending from 1976 to 2002), where 8275 new cases were reported in 1996[4]. Indeed, at that time, the control activities and follow-up treatments were seriously affected by the political instability and armed conflicts, resulting in an upsurge of new cases [15, 16].

[3] https://www.who.int/neglected_diseases/news/WHO-publishes-guidelines-treatment-sleeping-sickness/en/
[4] http://apps.who.int/gho/data/node.main.A1636?lang=en

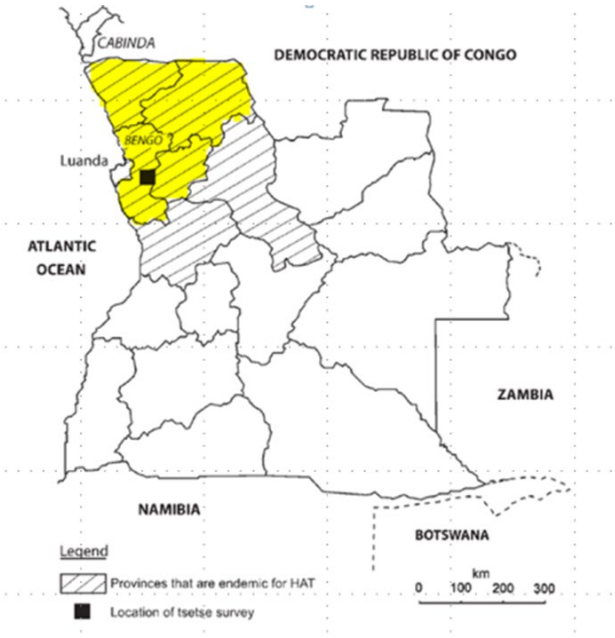


Figure 1-2: Northern trypanosomiasis endemic provinces of Angola. The seven endemic provinces are hatched. Highlightes in yellow are the 4 provinces where this work took place, namely Bengo, Zaire, Uíge and Kwanza Norte. Map adapted from: Truc et al., Annals of Tropical Medicine & Parasitology, Vol. 105, No. 3, 261.265 (2011).

The disease is typically found in rural areas with suitable habitats for the tsetse fly vector and frequent human-tsetse contact. Outdoor activities, like farming, fishing, hunting, collecting water, and washing clothes expose the people living in remote areas to the bite of an infective tsetse fly. Humans are the main reservoir for g-HAT in contrast to r-HAT, for which domestic and wild animals are the main reservoir.

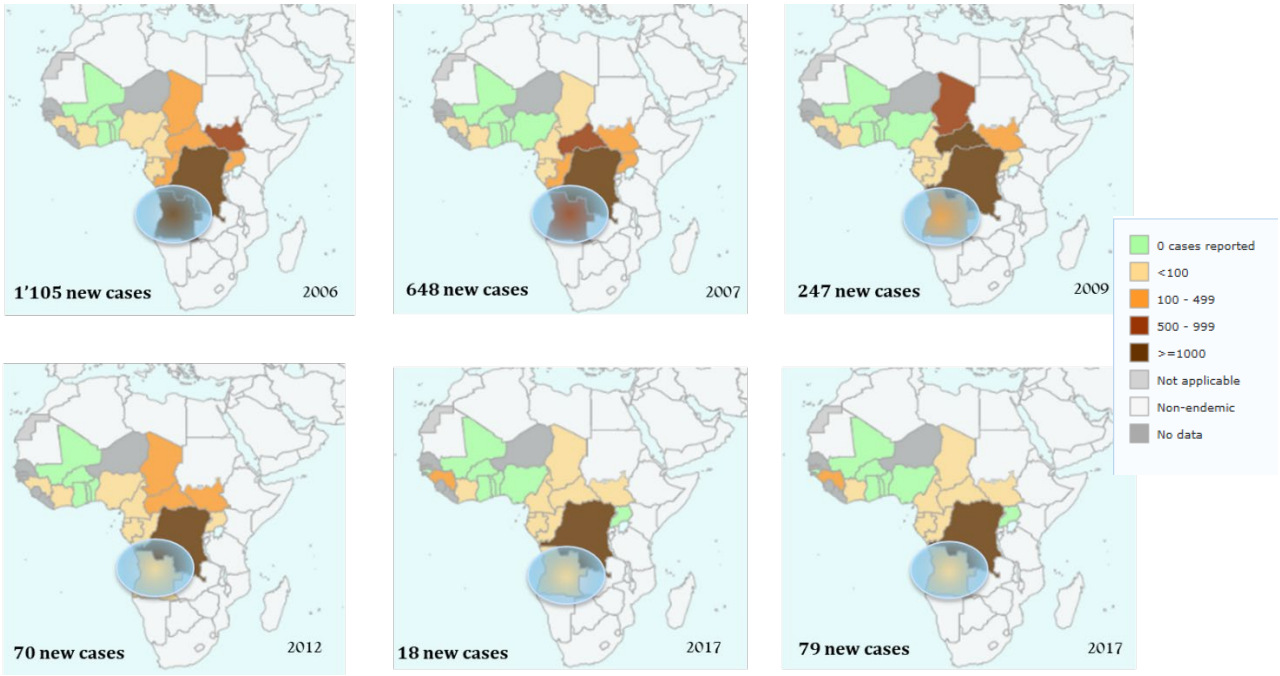


Figure 1-3: Incidence of trypanosomiasis from 2006 to 2018 in Angola. Data available from: http://apps.who.int/gho/data/node.main.A1636?lang=en.

1.2 African Traditional Medicine: its valorization and the scientific validation process

1.2.1 General aspects

Traditional medicine (TM), also called folk medicine (FM), ethno-medicine, native healing or complementary and alternative medicine (CAM) in western countries, is a culture-bound medical system to treat patients. Knowledge and practices are either codified in written, as for example in Traditional Chinese Medicine or in Ayurveda, or transmitted orally, as in the African Traditional Medicine (ATM). ATM is a non-codified, orally transmitted knowledge, generally held and used within a limited circle of people, as at community level cultural habits, treatment-seeking behavior favoring TM, fear of side effects produced by conventional treatment, or negative perception of modern health facilities are all reasons for the recourse to ATM. TM is thus broad and diverse. In such a multifaceted context, there is no single universal accepted definition of the term.

In this regard, it is worth to specify how "traditional" must be understood in the context of this work. In order to avoid any misconception, traditional medicine does not mean "old" but rather refers to a medicinal knowledge that has been, and constantly is re-producing knowledge, in a manner that it embodies the traditions of a people or community. "Traditional" refers, therefore, to an inter-generational knowledge, which is produced, maintained, modified, renewed, and developed collectively in order to make sense to the cultural understanding and representations of a community. As an example of the dynamics underlying the traditional knowledge, a study performed in two rural communities in Tanzania about malaria showed that traditional knowledge can integrate and/ or be integrated into recently acquired knowledge of biomedicine [17]. Thus, the collective, communal character of traditional knowledge is essential to its being "traditional."

Trying to give the most acceptable and consensual definition, the World Health Organization (WHO) provides the following definition: Traditional medicine is "*the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses*" [18]. Traditional healers, on the other hand are defined as "*a group of persons recognized by the community in which they live as being competent to provide health by using vegetable, animal, and mineral substances and other methods based on the social, cultural and religious backgrounds as well as on the knowledge, attitudes and beliefs that are prevalent in the community regarding physical, mental and social well-being and the causation of disease and disability"* [19]. This definition underscores two key elements of the traditional practitioners[5], i.e its holistic approach and the fact that traditional practitioners are accorded their status by the communities that they serve.

The single unifying theme in ATM is an enquiry into the causality of the illness. The patient consulting a traditional practitioner will rather ask after the reason (why am I suffering from?)

[5] According to the WHO: traditional practitioners are generally understood to be traditional healers, bone setters, herbalists, etc. *in* WHO Traditional Medicine Strategy 2002-2005, p.9. World Health Organization. Geneva. 2002

than after the cause (what am I suffering from?). In ATM practice, disease is considered to be due to several possible agents, the most important being:

- Natural causes, e.g seasonal changes, pathogenic agent
- Behaviour offensive to the patient's ancestral spirits, e.g the transgression of a social code
- Supernatural forces, e.g witchcraft

The role of the African traditional practitioner, apart from establishing the reason of illness and directing its cure, is also that of intermediary between the visible and invisible realities, religious, legal and political adviser and social worker. The accessibility and availability of qualified physicians is limited, with counts of only one medical doctor for 40'000 persons [20]. Hence, the traditional practitioner may be the only reliable source of relief. This explains why he plays an important role in the stability and social cohesion of the community, in which she or he lives.

Once the reason of illness has been disclosed, the traditional practitioner will prescribe the treatment, which may comprise only herbal remedies or call for additional measures, e.g behavioral adjustment or ritual procedures. In his holistic approach, the ATM meets the physical, mental and spiritual needs of the patient, so as health is achieved.

The herbal remedies used for the traditional treatment can arise from all plant parts: leaf, stem, bark, fruit, seed, root, flower, whole plant, and gums/resin. Generally, in rural areas, the traditional practitioner harvests his own plant material from natural stands of vegetation in the neighborhood, in contrast to one practicing in urban environment. In this latter case, either a herb seller or a travel to rural zones to replenish the stock of medicinal plants provides the plant material. Cultivation of medicinal plants in traditional medical practice is not widely practiced. One explanation may be that traditional practitioners consider cultivated plants less potent than their wild-grown counterparts. The collection of the plant material is often accompanied by rituals and a specific time of the day, of the year, or a particular stage of the plant's development, which are all traditional knowledge held by the practitioner. There are various traditional modes of preparation for the herbal remedy. However, aqueous infusions or decoctions are the most frequent forms. Other common modes of administration are inhalations, massages, direct consumption, and anal clysters.

1.2.2 Valorization of local knowledge and validation of African Traditional Medicine

The Alma-Ata Declaration by the International Conference on Primary Health Care (1978) was the first official recognition by the WHO and its member states of the role of the traditional practitioners and consequently of the traditional medicine in the primary health care. Indeed, in its will to achieve health for all through community participation, the WHO recommended the promotion of primary health care, as a response to the sanitary problems faced in low- and middle-income countries. In a long-term strategy, the primary health care strategy claimed to reach the largest number through in-depth work with local communities. By the establishment of a vast network of health units and agents capable of providing basic services of care as well

as promote health through education and hygiene, this strategy aimed at improving access to health for all. It is precisely in view of this major role that WHO recommended that traditional medicine, and eventually traditional practitioners, should be taken into account in the implementation of the Primary Health Care strategy. Thus, from then on traditional medicine became a partner of a renewed health policy.

In 2000, the Regional African Committee adopted the Regional Strategy on Promoting the Role of Traditional Medicine in Health Systems (Document AFR/RC50/9) [21], and an international guideline, on how traditional medicine should be evaluated was elaborated [22]. The Regional Strategy opened the way for the declaration by the Summit of Heads of States and Government of the first *Decade for African Traditional Medicine* (2001-2010). The adoption of this declaration in 2003 was a critical step in the recognition of the traditional medicine, its rational development and integration into the public health systems across Africa. Thus, during this decade, the African Traditional Medicine had to be promoted and integrated into national health systems in order to improve the health conditions of the population. The same year, the Africa Summit of Heads of States and Government declared the 31st of August as the African Traditional Medicine Day, in order to promote the role of traditional medicine in African national health-care systems. In 2013, the second *Decade of African Traditional Medicine* (2011-2020) was formalized and called for strengthening the implementation process of traditional medicine into public health systems, which requires, among others, generation of scientific evidence of the safety and efficacy of herbal remedies. An updated Regional Strategy on Enhancing the role of traditional medicine in health systems was elaborated covering a 10-year period (2014-2023) [23].

In response to all these resolutions and declarations, a number of African States recommended, generally as part of a redefinition of their health policy, the valorization of the traditional medicine. Many African countries developed the African Traditional Medicine to become a significant component of healthcare. The WHO Global Report on Traditional and Complementary Medicine 2019 summarizes the latest informations on the integrative approaches of the traditional and complementary medicine regarding policy, knowledge, and practice (see Figure 1-4) [24]. A recent survey analyzed the extent of the advance in research and development in the WHO African region [25]. The period 2000-2018 marked significant progress regarding the integration of ATM and traditional health practitioners into the national health systems. Several African countries (Benin, Burkina Faso, Cameroon, Ghana, Mali, Mozambique, Nigeria, Senegal, Uganda, United Republic of Tanzania) demonstrate exemplarity with regard to (i) policies and legal frameworks for traditional medicine practice, (ii) national programs dedicated to coordination of traditional medicine activities, (iii) the creation of National Expert Committees on African Traditional Medicine, (iv) the progress in R&D of Traditional Medicine, (v) the collaboration between traditional health practitioners and conventional health practitioners, and (vi) the integration of traditional health practitioners into the national health system. A prime example of a successful integration of traditional herbal products into the national list of the state-registered drugs is Ghana. This

country has at least 35 state-registered herbal products for the management of various diseases.

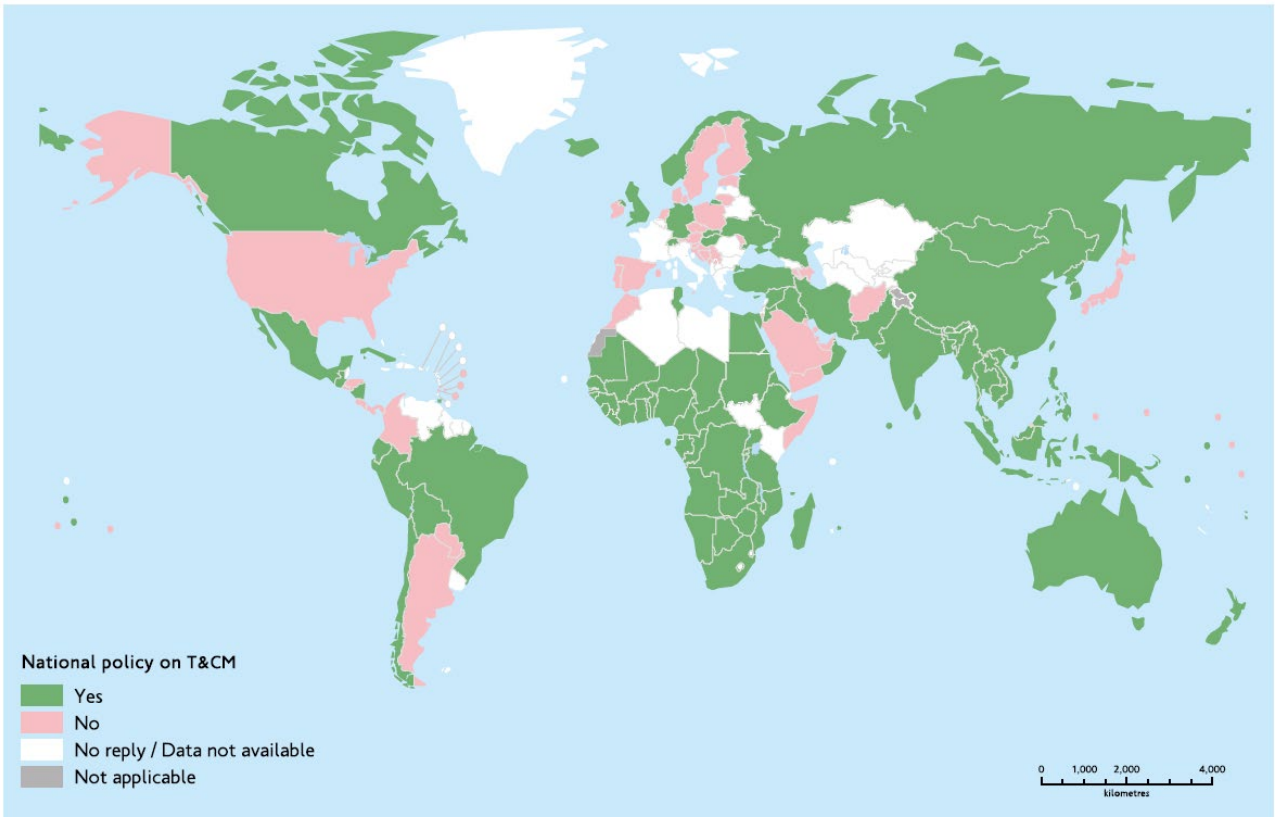


Figure 1-4: Member States with a national policy on traditional and complementary Medicine, 2018. (source: *WHO Global Report on Traditional and Complementary Medicine 2019*, WHO). Considering the situation in the WHO African region, of 47 Member States, 40 countries reported to have a national policy on TM and complementary Medicine as at 2018.

However, Ghana seems to be the exception rather than the norm in fully supporting the integration of traditional remedies and practices in the public health system. A mutual distrust between the conventional and traditional systems is observed. This may be explained by the insufficient in-depth examinations of the traditional methods and the efficacy as well as toxicity of its remedies, a great variation in the healing traditions, as well as the secrecy that often surrounds its practice.
By implementing regulation instruments for the use and the practice of African Traditional Knowledge, the valorization process has the objective to confer to this "heterogeneous" health system a standardized framework that aligns to the conventional one. The final aim is that valorization of traditional medicine provides safe and effective remedies in order to play a role as an alternative or complementary system in public health care.

In the context of R&D of TM, the valorization process requires scientific experimentation and scientific procedure to transform an oral knowledge into a written one. The WHO elaborated a set of tools and guidelines to support the scientific development of the Traditional Medicine [26]. WHO supports the member states by providing internationally acceptable guidelines and technical standards and also evidence-based informations. Relying on these

recommendations, the African States can formulate policies and regulations to control the safety, the efficacy, the quality and the access to traditional medicines.

Scientific Validation

The scientific assessment of traditional medicine is aligned with these international strategies and resolutions and aims to guarantee a safe and effective use of a herbal remedy through its clinical and pharmaceutical assessment. The objective of the scientific validation is to make traditional medicine an acceptable practice assessed with western-based biomedical standards in order to fulfill five criteria set by the WHO: safety, efficacy, quality, access, and rational use of its remedies. In that sense, the scientific promotion of traditional medicine targets the development of controlled herbal medicinal products.

The fundamental starting point of the validation process is the identification of the plant under study (see Figure 1-5). To select the most promising plant to treat a specific disorder, several approaches can be used, often in combination. First, a thorough ethnobotanical bibliographic review covering the use of plant of interest at local, regional, national and international level should be conducted[6]. After correspondence analysis, the most promising plants are selected. In case of paucity of scientific data in the country, an explorative study can be conducted to assess the use of the plants related to a specific disease. Another recommended approach is to run a Retrospective Treatment Outcome study (RTO-study) [27, 28]. The RTO-study is an observational study that enables a primary observation of the efficacy of the herbal preparation directly in humans.

Once the plant(s) of interest have been selected, plant extracts are prepared in order to proceed to pharmaco-toxicological assessment. This step requires *in-vitro* bioactivity testing, cytotoxicity evaluation, and *in-vivo* efficacy and toxicology investigations. Concerning the safety assessment, a primary risk of use is based on ethnographic data and toxicological profiles described in national pharmacopeia. In this regard, it is worth mentioning that one of the recommended "guiding principle" of the WHO in assessing herbal medicines is that if the remedy "has been traditionally used without demonstrated harm, no specific restrictive regulatory action should be undertaken unless new evidence demands a revised risk-benefit assessment" [29]. In parallel, phytochemical analyses are run in order to isolate and identify the active constituents in the plant under investigation. The active components can serve as marker for the quality assessment of the raw plant material. The successful plant candidate demonstrating convincing pharmaco-toxicological results will undergo morphological evaluation to establish quality control criteria for the raw drug material. Further, large clinical trials are carried out in order to confirm the reliability of the pre-clinical results. However, proof of safety and efficacy should take into account the well-established uses of a herbal

[6] On this topic, the *Research Initiative on Traditional Antimalarial Method* (RITAM) in its efforts to select the most promising plants used against malaria has developed a ranking system, called the "Important Value for the treatment of Malaria" (IVmal). This system enables to assign an "IVmal" according to how widely they are used for the treatment of malaria. The hypothesis underlying this ranking method is that a plant that is widely used and reported against malaria in three different continents must have a higher probability to demonstrate antimalarial properties.

remedy. At the end of the validation process, the research should lead (i) to confirm or reject the relevance of the plant for the studied indication and (ii) to discover other interesting effects for the expansion of its therapeutic indications. As so, validating traditional medicine is producing enough evidence of its safety and efficacy.

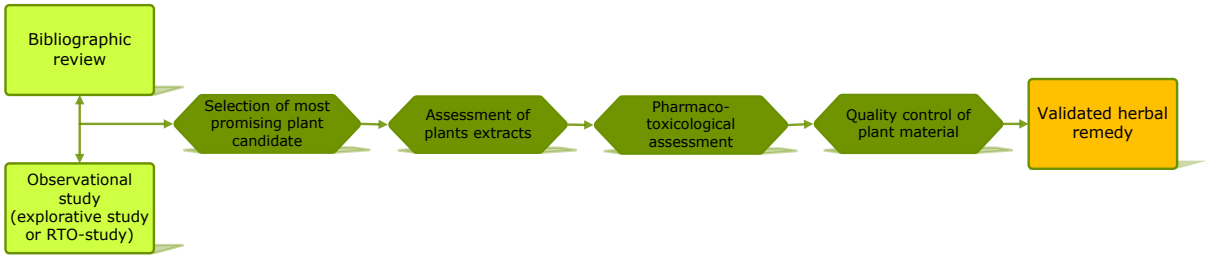


Figure 1-5: Simplified flowchart of the validation process of an herbal remedy.

The scientific evaluation of the traditional remedies is a long-term process including different fields of expertise. Frequently that the competent national institute leading ethnopharmacological research to validate and promote the traditional herbal preparations is embedded in a highly collaborative south-south or south-north network in order to achieve this multidisciplinary work.

Beyond the scientific assessment, the validation process allows the transcription of an oral knowledge, that otherwise is destined to be transformed or in even to disappear. The publication of traditional knowledge and its associated practices constitute a form of recognition and valorization. By being published, traditional knowledge becomes public knowledge, which can be preserved, protected and maintained. A good illustration of the role of the scientific validation and its impact on research and preservation of the traditional knowledge is the TRAMIL[7] program in the Caribbean. TRAMIL is an interdisciplinary program led in the detection, validation and diffusion of the uses of medicinal plants that impact in primary health care.

A disease that deserves special attention in relation to validation of traditional medicine is malaria. Indeed, many studies have been carried out to assess the antiplasmodial potential of traditional remedies [30]. The reason is that the *Cinchona* tree and *Artemisia annua* plant have provided the basis for two of the three main classes of antimalarials, thus opening the doors of hope that other plants will be found as effective antimalarial agents. Despite the plethora of pre-clinical studies conducted on the antimalarial potential of medicinal plants, only few clinical trials on safety and efficacy of traditional antimalarials have been carried out [31-35]. An example of a traditional remedy that proved its effectiveness against malaria is *Argemone mexicana* L. The plant was selected initially through a RTO-study conducted in Mali among local population, because all the involved informants reported that consumption of the decoction of the plant was always followed by a complete cure in case of uncomplicated malaria [27, 36, 37]. Based on these good results, a dose-escalating prospective study and a prospective randomized controlled trial were organized. Both clinical trials gave very

[7] See http://www.tramil.net/en

promising results with a dose-response effect and a stringent reduction of severe cases [38, 39]. *Argemone mexicana* is today a government-approved phytomedicine against malaria called "Soumafoura Tiemoko Bengaly" [40]. This improved traditional medicine (ITM) was even proposed as a complement to standard drug treatment in high transmission areas to fight resistance to artemisinin combination therapy [41].
More recently, *Terminalia macroptera*, a Malian plant used traditionally to treat a large variety of diseases including malaria, has passed the hurdle of the *in vivo* pharmacological assessments. After several complementary investigations, the plant should be proposed in the future as an ITM entering the Malian phyto-industry [42, 43]. Another registered and marketed ITM in Mali is "Malarial-5®", which is composed of a decoction of *Senna occidentalis, Lippia chevalieri*, and *Acmella oleracea* [44]. Other doctoral theses illustrate, how academia can contribute to the scientific validation of traditional remedies [45, 46].

Compared to malaria, trypanosomiasis received less attention in relation to traditional medicine. Indeed, a Pubmed database search with the keywords "traditional medicine & trypanosomiasis" and "traditional medicine & malaria" returns 75 and 1224 references, respectively.
Among the scientific articles on the antitrypanosomal potential of African medicinal plants [47-50], some have identified encouraging *in vivo* anti-trypanosomal activity. We can appoint *Kaya senegalensis,* whose stem bark aqueous extract was active against *Trypanosoma brucei brucei* inducing 90% inhibition with an effective dose of 100-300 mg/kg [51]. Another highly cited plant is *Annona senegalensis Pers.*[52]. Recently, a group of ten quinoline alkaloids isolated from *Waltheria indica* showed promising activity against *Trypanosoma cruzi*, displaying IC_{50} values lower than the reference drug benznidazole [53].
However, concerning African trypanosomiasis, only one natural compound has so far shown promising activity, namely cynaropicrin [54]. This sesquiterpene lactone preferentially inhibited *T. b. rhodesiense* (IC_{50} of 0.3 µM) and *T. b. gambiense* (IC_{50} of 0.2 µM). When injected intraperitoneally in the *T. b. rhodesiense* STIB 900 acute mouse model, this active compound led to a 92% reduction of parasitemia compared to untreated controls on day seven post-infection [54]. However, no natural compound has so far shown in vivo activity in stage 2 in vivo models of infection.
To the best of our knowledge, no scientific validation was undertaken assessing a traditional preparation used in the management of trypanosomiasis. This might be due to the fact, that trypanosomiasis is a lethal disease, if left untreated, and its symptomatology is complex and not evident. It could also be explained by the fact that there is no traditional herbal remedy known to treat this specific affection, but rather herbal remedies used to alleviate some of its accompanying symptoms.

1.2.3 Traditional Medicine and its practice in Angola

In response to all the resolutions and declarations by the WHO, Angola has created a department dedicated to the traditional medicine within the National Institute of Public Health, renamed in 2018 as Institute of Health Investigation (*Instituto de Investigação em Saude*). However, to the best of our knowledge, the research unit dedicated to the traditional

medicine is not conducting effective ethnopharmacological research on traditional medicine due to the lack of equipped laboratories and a trained scientific team. According to the recent work of Kasilo et al. on the advances and development of traditional medicine in Africa, Angola has a national program for traditional medicine implemented in the Ministry of Health [25]. In this study, of 47 countries analyzed, Angola together with Algeria, Botswana, Cape verde and South Sudan, belongs to the 5 least advanced countries regarding the progress on integration of Traditional Medicine in the national health system. Angola elaborated in 2010 an exclusive national traditional medicine policy, which has not been so far adopted by the parliament. Therefore, traditional medicine is not formally approved by the public health policy; however, its practices are somehow accepted. There is no system for the official approval of the traditional medical practices and remedies. Thus, one could say that the Angolan public health system is tolerant[8] toward traditional medicine and the use of traditional herbal products is not forbidden, but tolerated. In 2012, the 1st National Conference of Traditional Medicine and Complementary Practices was organized in Luanda. This event was a first sign of the Angolan political authorities to make a step towards the recognition and integration of traditional practices in the health system.

In Angola 72, 4%[9] of the population uses herbal medicine[10] to treat various medical affections, including parasitic infections such as Human African Trypanosomiasis [HAT]. The herbal preparation is either recommended by a traditional practitioner or taken as a self-treatment. The plant material is collected directly from the wild or bought on markets. One of the main herb market in Luanda is called “Mercado dos Kwanza”.

There are three main traditional practitioner associations, namely the Professional Chamber of Traditional, Alternative and non-Conventional Practitioners in Angola (CATEMETA), the National Council of Traditional Natural Medicine in Angola (CONMENTA), and the Union of Traditional Practitioners in Angola (UTTDA). The CATEMETA association constitutes the most important one across the country, with more than 61’000 members. Membership is granted through approval by the directory board of the association. However, the adhesion criteria remain quite unclear and there is no evaluation process of their competencies, and neither a national registration nor a licensing procedure.

[8] According to WHO: “in countries with a tolerant system, the national health care system is based entirely on allopathic medicine, but some TM/CAM practices are tolerated by law”, in WHO Traditional Medicine Strategy 2002-2005, p.9. World Health Organization. Geneva. 2002-

[9] Percentage stated at the 1st *National Conference of Traditional Medicine and Complementary Practices* held in Luanda August 2012

[10] According to WHO a herbal medicine is defined as “finished, labelled medicinal products that contain as active ingredients aerial or underground parts of plants or other plant material, or combinations thereof, whether in the crude state or as plant preparations. Plant material includes juices, gums, fatty oils, essential oils, and any other substances of this nature. Herbal medicines may contain excipients in addition to the active ingredients. Medicines containing plant material combined with chemically defined active substances, including chemically defined, isolated constitutes of plants, are not considered to be herbal medicines. Exceptionally, in some countries herbal medicines may also contain, by tradition, natural organic or inorganic active ingredients which are not of plant origin” (see WHO, Guidelines for the assessment of herbal medicines, 1991). However, in this thesis work, the expression herbal medicine refers to a herbal preparation, which has not been yet validated thus isn’t so far a finished and labelled product.

In terms of category, there are three main types of traditional practitioners: (i) the herbalist ("curandeiro"), (b) the spiritualist ("espirita") or (iii) the sorcerer ("kimbandeiro"). Considering the legal framework, the traditional practitioners and the associated traditional knowledge have no legal status, thus compromising their protection and the preservation of the Traditional Knowledge.

1.3 Research aims and outline of the thesis

As evidenced by the diverse themes described in the introduction, the valorization of African Traditional Medicine through its scientific validation is by nature an interdisciplinary task. For this thesis, different fields of competences were brought together in a collaborative manner, in order to pursue the various scientific activities of the validation process. The common thread across the chapters is the exploration of the medicinal plants used in Angola against trypanosomiasis. There is a lack of information on Angolan medicinal plants currently used for the treatment of this disease. The thesis goal is to fill up this gap by delivering useful information regarding the usage of the traditional medicines and to contribute to the scientific validation of the most promising herbal preparations used to treat g-HAT.

In **Chapter 2**, the compliance challenges regarding ethical, Intellectual Property Rights (IPR), and Access and Benefit-Sharing requirements are exposed and discussed. Indeed, the implementation in Angola of the field activities on Traditional Knowledge associated to Genetic Resources required to be compliant with the IPR and ABS regulations.

In **Chapter 3**, the use and access to the Angolan traditional medicine for the management of trypanosomiasis was investigated, by conducting an explorative study in four endemic provinces of Angola. It was hypothesized that persons living in endemic rural areas with no direct access to reference medication nor health facilities would turn to folk medicine before referring to a conventional treatment. Together with the research team, 30 patients and 9 traditional practitioners were surveyed and the reported plants were collected. Using an ethnomedical and ethnobotanical approach, the accessibility to folk medicine, the local therapeutic practices as well as the plant species used were analysed. Primary recommendation in terms of toxicity of the plants used was discussed.

In **Chapter 4**, nine plant candidates were selected out of the previously reported plants for further investigation. It was expected that from this panel, the most promising traditionally used plant could be determined. The selection of various active plant extracts through screening activities together with bioguided-fractionation enabled to identify a promising local recipe and to isolate and characterize several active constituents.

1.4 General Methodology and Partnerships

The overall project was elaborated in a manner to be a collaborative research work, whose scientific activities were divided between Angola and Switzerland (see 1.4.1). The project included Swiss and Angolan stakeholders, both non-academic and academic ones. Within the framework of this thesis work, six Angolan students and one Swiss student had the opportunity to participate and carry out their bachelor thesis.
In all, 10 collaborative entities were integrated in this research project (see 1.4.2)

1.4.1 Overview of the research methodology

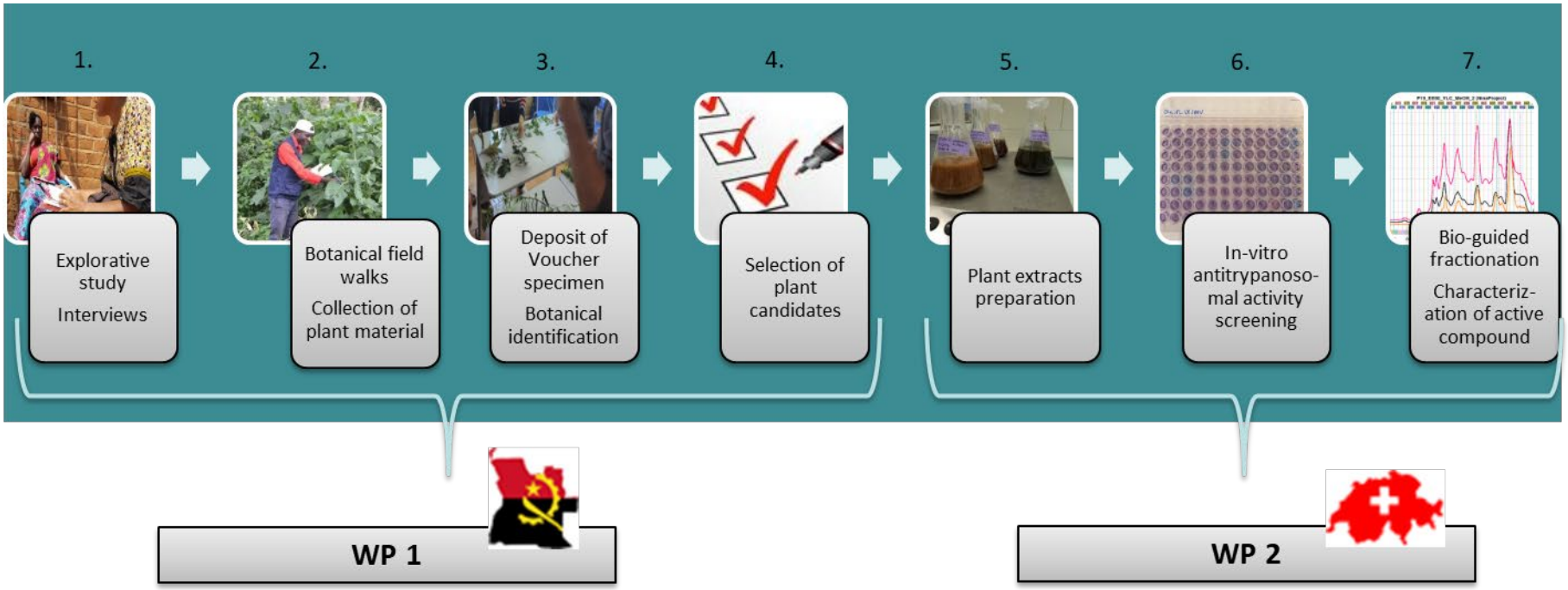


Figure 1-6*:* Overview of the different steps (1-7) of the research project split in two workpackage (WP). WP 1 was performed in Angola and WP2 in Switzerland.

WP1 started in Angola with step 1, which corresponded to an explorative study among traditional practitioners and patients suffering from trypanosomiasis. This first step integrated five Angolan bachelor students in medical anthropology. In step 2, botanical field walks with the informants enabled to collect the voucher specimen of each reported plant. Step 3 was the botanical identification of the collected plant species. This step integrated an Angolan bachelor student in botany, so as he could perform his bachelor thesis in the framework of this research. A voucher specimen of each species was deposited in a reference herbarium. Step 4 corresponded to the selection of the most promising plant candidates using mainly 4 criteria: the use report, the scientific available data on the species, the qualitative aspect of the reported information, and the novelty of the plant.

WP2 started in Switzerland with step 5. Plant extracts from the preselected species were produced with different solvents. Step 6 corresponded to the *in vitro* antiprotozoal activity screening and cytotoxicity determination. It enabled the selection of the most promising active extracts. During step 7, the selected active extracts were fractionated in order to isolate and identify the active constituents.

1.4.2 The collaborative network

Collaborative Entity	Contact person	Function
Ministry of Science and Technology	Ex-Minister of Science and Technology in Angola: Prof. Dr. Maria Cândida Pereira Teixeira	Funding Agency
National Center for Scientific Research (CNIC)	General Director of CNIC, Prof. Dr. Pedro João Guilherme	Logistic and administrative support
Institute of Fight and Control of Tripanosomiasis (ICCT)	General Director of ICCT, Prof. Dr. Josenando Théophile	Scientific medical expert in control of tripanosomiasis
National Insitute of Biodiversity and Conservation Areas (INBAC)	General Director, Dr. Aristófanes Pontes	National competent authority who grants access to Genetic Resources and negotiate the terms of the Mutually Agreed Terms
National Focal Point	Msc. Elizeth Gonçalvez	Guidance on conditions for access to Genetic Resources and procedures to observe
University of Agostinho Neto, Faculty of Social Sciences, Department of Anthropology	Head of Department, Prof. Dr. Luzia Milagre	Allocates human resources (BSc students) for field work; selects students to perform their bachelor work related to the research project
University of Agostinho Neto, Faculty of Sciences, Center of research in botanical studies	Director of the Center, Prof. Dr. Esperança da Costa Maria Eduardo Francisco da costa	Scientific expertise in botanics; mobilizes students to perform their bachelor work as part of the research project
Professional Chamber of alternative, non-conventional and natural traditional practitioners (CATEMETA)	President and coordinator: Kitoko Maiavangua	Collaboration with traditional practitioner members of CATEMETA
National Council of Traditional Natural Medicine in Angola (CONMENTA)	President , Dr. Adriano José Manuel	Collaboration with traditional practitioner members of CONMENTA

Phytopharmacy and Natural Products research group, Zürcher Hochschule für Angewandte Wissenschaften, Wädenswil (ZHAW)	Head of the research group, Dr. Evelyn Wolfram	Scientific expertise in phytopharmaceutical methodology; hosts HPLC bioguided fractionation and HPTLC toxicity profiling
Phytochemistry and Bioactive Natural Products research group, Swiss Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva (ISPSO)	Head of the research group, Prof. Jean-Luc Wolfender	Expertise in phytochemical analysis; allocates lab space, instruments, and a highly skilled team

2 VALIDATION PROCESS OF HERBAL REMEDIES AGAINST TRYPANOSOMIASIS: ADDRESSING NATIONAL AND INTERNATIONAL COMPLIANCE - A CASE STUDY FROM ANGOLA

Nina Vahekeni[a,b], Pedro João Guilherme[c], Pascal Mäser[a,b], Peter van Eeuwijk[a,b]

[a]Swiss Tropical and Public Health Institute (Swiss TPH), Socinstr. 53, 4051 Basel, Switzerland

[b]University of Basel, Petersplatz 1, 4001 Basel, Switzerland

[c] National Institute of Scientific Investigation (CNIC), Luanda, Angola

Working manuscript, December 2019

Abstract

In the framework of an ethnopharmacological research study, a collaboration between a Swiss academic Institute and an Angolan national research institute was established. The ethnobotanical part was carried out in Angola and the laboratories investigations in Switzerland. In this research context, accessing Genetic Resources and associated Traditional Knowledge for investigation purpose requires to align with international treaties and domestic legislation related to the Access and Benefit-Sharing principles. Moreover, it also had to consider the ethical requirements for clinical studies and the Intellectual Property Rights of the holders of the traditional knowledge.

This case study questions the compliance aspects related to ethical, Intellectual Property Rights, and Access and Benefit-Sharing principles in the case of a scientific collaboration between a *User* country (Switzerland), which is party to the Convention on Biological Diversity and the Nagoya Protocol and a *Provider* country (Angola), which is only party to the Convention on Biological Diversity at the time of the field activities.

This case study outlines how compliance could be achieved in the framework of a research on scientific validation of traditional medicines used in Angola against trypanosomiasis.

2.1 Introduction

This research project aimed at validating local herbal preparations used in the management of sleeping sickness in Angola. The validation process requires (i) gathering ethnobotanical information on the use of the herbal remedies and (ii) assessing the biological activities (as well as toxicity) of the selected medicinal plants. In our case, the ethnobotanical research activities were carried out in Angola; the phytochemical and pharmacological activities in Switzerland. Thus, the project's scientific activities were distributed between these two countries. The ethnobotanical part started with interviews among traditional practitioners and infected persons (patients), with the objective to collect traditional knowledge on the use of medicinal plants. This first activity ran over a 5-month period, from October 2016 to March 2017. It was followed by the collection of some of the reported plant species, which took place from February 2018 to April 2018. The plant material was then exported to Switzerland, in order to proceed to laboratory research. In this context, accessing Genetic Resources (GR) and associated Traditional Knowledge requires to align with international treaties and domestic legislation related to the principles of Access and Benefit-Sharing (ABS). In this respect, the project had to be fully compliant with the Swiss and the Angolan legal, administrative and regulation measures regarding the ABS framework. Moreover, it also had to consider the ethical requirements for clinical studies and the Intellectual Property Rights (IPRs) of the traditional practitioners involved in the project. The complexity of national and international regulations concerning ethical, IPRs and biodiversity requirements is another barrier, which must not be underestimated. The main question to be resolved was *how to be compliant when accessing genetic resources and associated traditional knowledge in a country that (i) became party to the Nagoya Protocol during the field activities of the project and (ii) is lacking a domestic ABS regulation system*? Responding to the compelling need for evidence regarding traditional medicines, this case study outlines how compliance regarding ethical, IPRs and ABS requirements at national and international level was fulfilled in order to conduct the first ever research on validation of traditional medicine used in Angola.

2.2 Study background

In the context of ethnopharmacological field studies, two main international treaties regulate the utilization of genetic resources: *the Convention on Biological Diversity* (CBD)[55] and the *Nagoya Protocol* (NP[56]. The CBD, signed in 1992, addresses questions related to the conservation and sustainable use of biological diversity. In its Art. 15, it defines the three key elements of the ABS mechanism, namely the *Mutually Agreed Terms* (MAT), the *Prior Informed Consent* (PIC), and the *Fair and Equitable sharing of the benefits* arising out of the utilization of genetic resources. The Nagoya Protocol entered in force in 2014 and is a supplementary agreement to the CBD, aiming to facilitate the implementation of the ABS procedure. Thus, the Nagoya Protocol establishes between the parties (the *Provider* and the *User* of the genetic resources) contractual obligations on access, benefit-sharing, and compliance that are reflected in the two binding measures, the MAT and the PIC. For our purpose, Angola was the *Provider* of the GR and Switzerland the *User*. Moreover, the NP defines National Focal Points and Competent Authorities as institutional structures to enable procedural requirements.

Additionally, it establishes an internet-based portal, the ABS Clearing House[11], which is an exchange platform aiming at facilitating access to information on ABS. To date (November 2019), the Nagoya protocol has been ratified by 123 countries. Switzerland ratified the Nagoya Protocol on July 11th 2014[12], Angola is party to the Nagoya Protocol since May 7th 2017[13]. According to Art. 15/1. of the Nagoya Protocol, it is up to the parties to transcribe the ABS measures into individual laws and regulations as well as to implement them at national level. In Switzerland, the NP came into force on 12 October 2014 with the Natural and Cultural Heritage Protection Act [57]. The related Nagoya Ordinance (NagO), entered in force on 1 February 2016 [58]. In its Art. 3, 4, and 5 are the provisions that Swiss researchers must satisfy when accessing GR and associated traditional knowledge of countries that are Parties to the NP. Angola has not yet translated the NP into a national law, and the ABS procedure has so far not been implemented into a domestic regulation system. However, the different institutions granting access to the GR follow their own administrative measures in order to best apply the ABS procedure [59].

Even if a country lacks formal ABS regulation, ethical principles need to be respected. This is frequently neglected in ethnopharmacological studies, for which a lack of ethical compliance has been observed [60]. The study protocol of our research project had to follow both Swiss and Angolan ethical requirements. In Switzerland, 7 different Ethics Committees regulate the ethical aspects of the research activities[14]. In our case, the project being attached to the Swiss Tropical and Public Health Institute located in the northwestern part of Switzerland, ethical clearance had to be given by the Ethics Committee of Northwestern and Central Switzerland (EKNZ). On the Angolan side, the creation of the first medical ethical committee dates from March 2000, driven by the necessity to approve a clinical trial on trypanosomiasis, which was led by the Swiss TPH in collaboration with the National Institute for Fight and Control of Trypanosomiasis (ICCT)[61]. Later on, a national Ethics Committee was constituted and affiliated to the National Public Health Institute. In addition to the approval of the Ethics Committee, the professional ethical code of the *International Society of Ethnobiology* (http://ethnobiology.net/code-of-ethics/) as well as the international law and ethical principles on rights of indigenous people [62] had to be considered.

In its Art. 31, the *United Nations Declaration on the Right of Indigenous Peoples* points out that indigenous people have the right to "control, protect and develop their intellectual property over […] traditional knowledge". Moreover, the States shall "take effective measures to recognize and protect the exercise of these rights". Tackling the question of Intellectual Property Rights (IPRs) and the status of Traditional Knowledge (TK), Angola has recognized within the *Law on Cultural Patrimony* in its Art.45/4 and 46/b that Traditional Knowledge associated to medicinal plants is a Cultural Good that shall be protected by the State by

[11] See ABS Clearing House website: https://absch.cbd.int/
[12] See country profile on the CBD website: https://www.cbd.int/countries/?country=ch
[13] See country profile on the CBD website: https://www.cbd.int/countries/?country=ao
[14] See swissethics website: https://www.swissethics.ch/eks_e.html

different means, among others by promoting the protection of the holders of the Traditional Knowledge[15].

One question remains: *How to define the holders of TK*? Even if traditional practitioners and traditional knowledge are defined in the law as being part of the Angolan cultural heritage and shall be protected, the mechanism in force to define, recognize, and protect the TK and the traditional practitioners remains unclear. Moreover, neither in the CBD nor in the NP there is a definition of the concept of indigenous and local communities and traditional knowledge[16]. Nonetheless, without a clear definition, the delimitation of the community and the identity of its members is problematic. This legal grey area prevents from identifying and protecting the holders of the TK. Moreover, it compromises the fair distribution of the benefits arising from the research among its beneficiaries.

2.3 Research methodology

The scientific validation of the herbal preparations used in the management of trypanosomiasis was performed in 5 phases. Several partnerships at national and international level were established along these 5 phases in order to fulfill ethical, IPRs and ABS compliance. Figures 2-1 to 2-5 summarize for each phase the different collaborations and scientific activities. Considering that Angola is Party to Nagoya Protocol since 7th May 2017, it is worth mentioning that phases 1 (from May 2015 to June 2016) and 2 (from October 2016 to March 2017) were not subject to the ABS measures imposed by the Nagoya protocol.

Phase 1: Consolidation of the project into the Angolan research landscape (May '15 – June '16)

In the first quarter of 2015, the Swiss-Angolan principal investigator (PI) was integrated as an auxiliary collaborator to the scientific panel of the National Center of Scientific Investigation (CNIC), and the project's research activities were included into the agenda of the CNIC. Mid of 2016, a first collaborative agreement (see Appendix / Document 1) was established between the CNIC and the Institute of Combate and Control of Trypanosomiasis (ICCT) in order to enable the implementation and the field activities of phase 2.

[15] See: Law n° 14/05 from the 7 th October 2005; https://wipolex.wipo.int/fr/legislation/details/11009

[16] In the absence of national operational tools to regulate the IPR on Traditional Knowledge, Angola provides a legal framework by aligning its IPR policies to international regulation texts of World Intellectual Property Organization (WIPO).

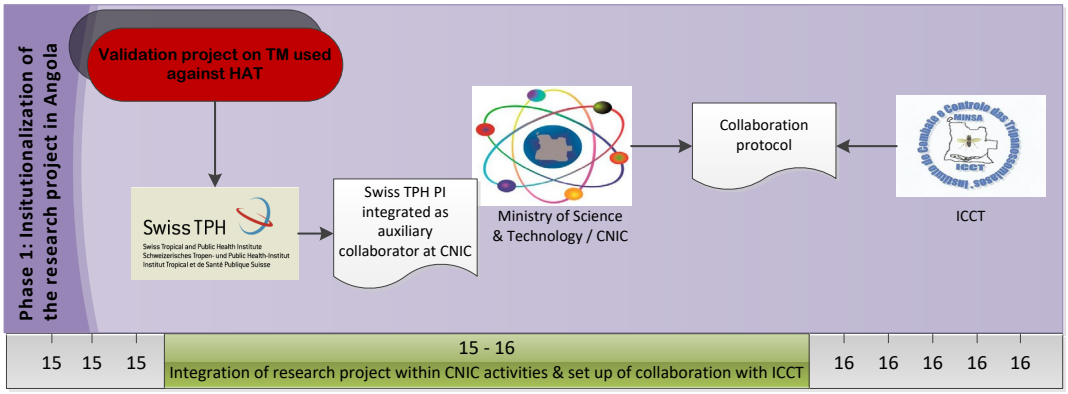


Figure 2-1: Phase 1 Consolidation of the research project in the Angolan research landscape.
The validation project on traditional medicine (TM) used against sleeping sickness or Human African Trypanososomiasis (HAT) was integrated to the CNIC scientific activities. Then a collaboration was established between the CNIC and another national institution, the ICCT. This alliance gave to the project a solid basis and recognition in the Angolan scientific landscape. The green bar indicates the duration of the scientific activities. Swiss TPH: Swiss Tropical and Public Health Institute.

Phase 2: Field study implementation (October '16 – March '17)

Phase 2 was dedicated to the investigation of traditional knowledge related to the use of medicinal plants in the management of sleeping sickness. Two groups of informants were inquired: traditional practitioners and patients (who were under follow-treatment under responsibility of the medical team of the ICCT). Phase 2 included two main scientific activities: (1) the ethnobotanical field study, which aimed at gathering information on the usage of herbal preparations from the traditional practitioners and patients; (2) the collection of the reported plants in the field. The implementation of these two scientific steps required several partnerships. First, the pre-established-phase 1 collaboration between the CNIC and the ICCT enabled the PI to access ICCT's medical files of the patients, to localize them and be supported by the ICCT team for logistic as well as administrative activities. Second, in order to access the traditional knowledge in hands of the traditional practitioners, an agreement was concluded with the board of the two main traditional practitioners' national associations, namely the Professional Chamber of Traditional, Alternative and non-Conventional Practitioners in Angola (CATEMETA) and the National Council of Traditional Natural Medicine in Angola (CONMENTA); see Appendix 1/ Document 2 and 3. This agreement allowed the PI, in collaboration with the scientific leaders of the CATEMETA and the CONMENTA as well as with the correspondent provincial directors, to select 14 traditional practitioners to be recruited into the study. In addition, two academic collaborations with the University of Agostinho Neto (UAN) were established with the aim to respond to the third pillar of the ABS mechanism, benefit sharing. The first partnership took place between the Swiss TPH and the Department of Anthropology of the Faculty of Social Sciences, and the second one between the CNIC and the Center of Studies and Scientific Investigation on Botanic of the Faculty of Sciences (see Appendix 1/ Document 4 and 5).

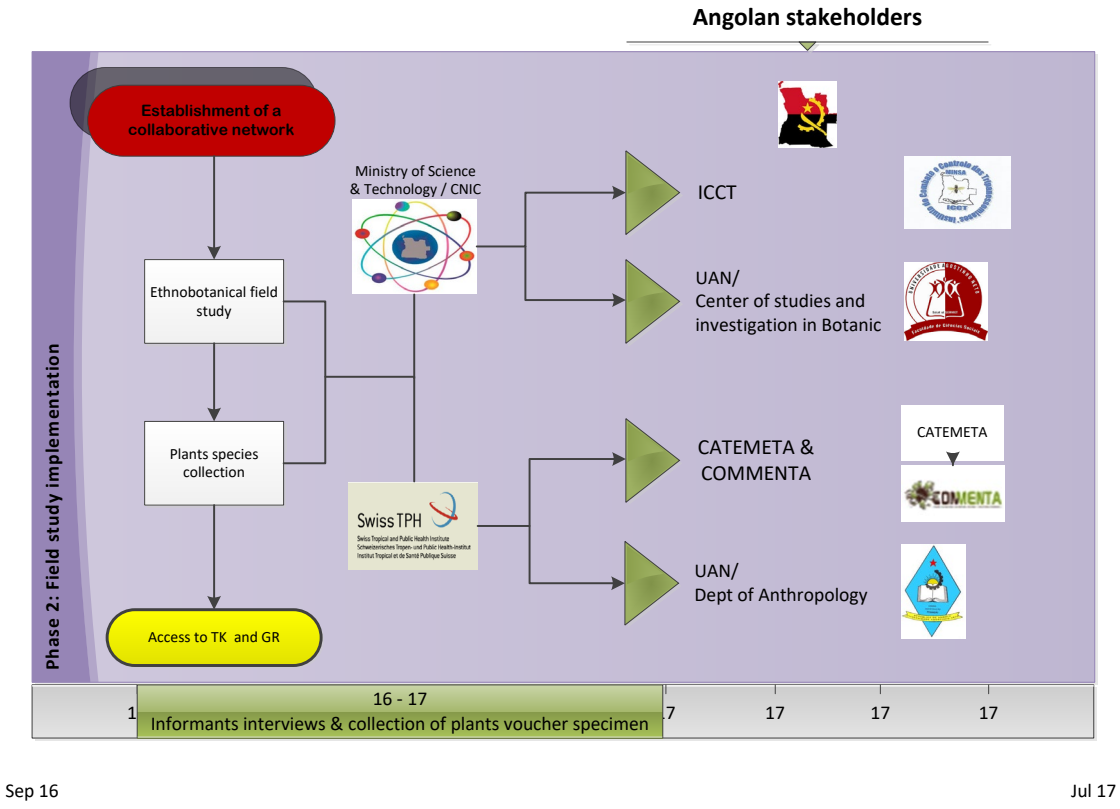


Figure 2-2: Phase 2 Field study implementation
The establishment of the collaborative network was a prerequisite to implement the field study work. The collaborative network included, in addition to the Swiss TPH, 6 different entities: the CNIC, the ICCT, the CATEMETA, the CONMENTA, the Department of Anthropology, and the Center of Studies and Scientific Investigation in Botany. The scientific network guaranteed the realization of the two main scientific activities of phase 2: the ethnobotanical field study and the collection of plants. Thus, the collaborative network enabled accessing the Traditional Knowledge (TK) and the associated genetic resources (GR).

Phase 3: Collection and exportation of plant material (May '17 – April '18)

Phase 3 started in May 2017, the date from which on Angola became Party to the Nagoya Protocol. Consequently, the collection and export of plants fell under the ABS requirements.

The collaboration with the Center of Studies and Scientific Investigation on Botany provided expertise on plant species identification. As required for ethnobotanical studies, a voucher specimen of each identified species was deposited in the Center of Studies and Scientific Investigation on Botany, a national botanical reference center. Once the plant species had been identified, several plant candidates were selected based on literature review for further laboratory investigations to be conducted in Switzerland. Thus, a certain amount of plant material had to be collected. Accessing genetic resources associated to traditional knowledge was subject to the ABS requirements of the newly signed Nagoya Protocol. At that time, the National Institute of Biodiversity and Conservation Areas (INBAC) was the designated national authority in charge to negotiate the *Prior Informed Consent* and the *Mutually Agreed Terms.* In addition, a National Focal Point had also been indicated[17]. Thus, for the negotiation process of

[17] The INBAC and the name of the National Focal Point were given on the former version of the website of the ABS Clearing House

the ABS agreement, a collaborative work was established with the direction of the INBAC and the National Focal Point, who was employed by INBAC at that time. Moreover, in compliance with the international regulations regarding the international trade of endangered species, the Angolan administrative authority of the *International Convention on the Trade with endangered Species of Wild Fauna and Flora* (CITES) was consulted.

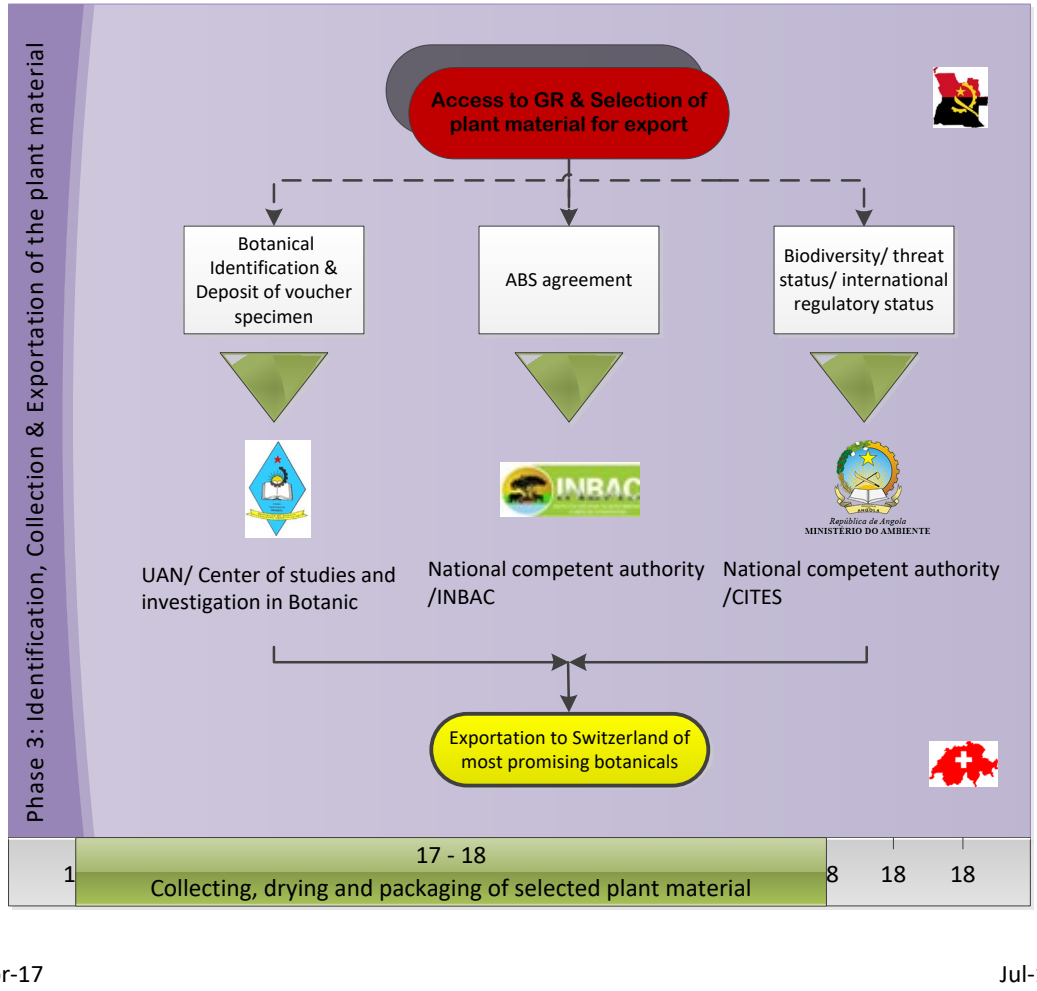


Figure 2-3: Phase 3 Identification, Collection & Exportation of the plant material
The export of the plant material required 3 steps: (1) confirmation of the botanical identity of the collected plant, (2) procurement of the PIC and the negotiation of the MAT with the INBAC and National Focal Point, (3) procurement of the national export permit. Three collaborative bodies were implicated: The Center of Studies and Scientific Investigation in Botany, the INBAC, and the administrative authority of the International Convention on the Trade with endangered Species of Wild Fauna and Flora (CITES).

Phase 4: Antiparasitic activity screening and isolation of active compounds (June '18 – October '19)

All screening activities and phytochemical analyses to characterize the active constituents of the plant extracts were carried out in Switzerland. Two Swiss academic entities were included besides the Swiss TPH, the Phytopharmacy and Natural Products group of the Zurich University of Applied Sciences (ZHAW) and the Phytochemistry and Bioactive Natural Products group of the Swiss Institute of Pharmaceutical Sciences of Western Switzerland

(ISPSO) of the University of Geneva. In terms of ABS compliance, these two research groups were considered as Third Parties accessing the genetic resource.

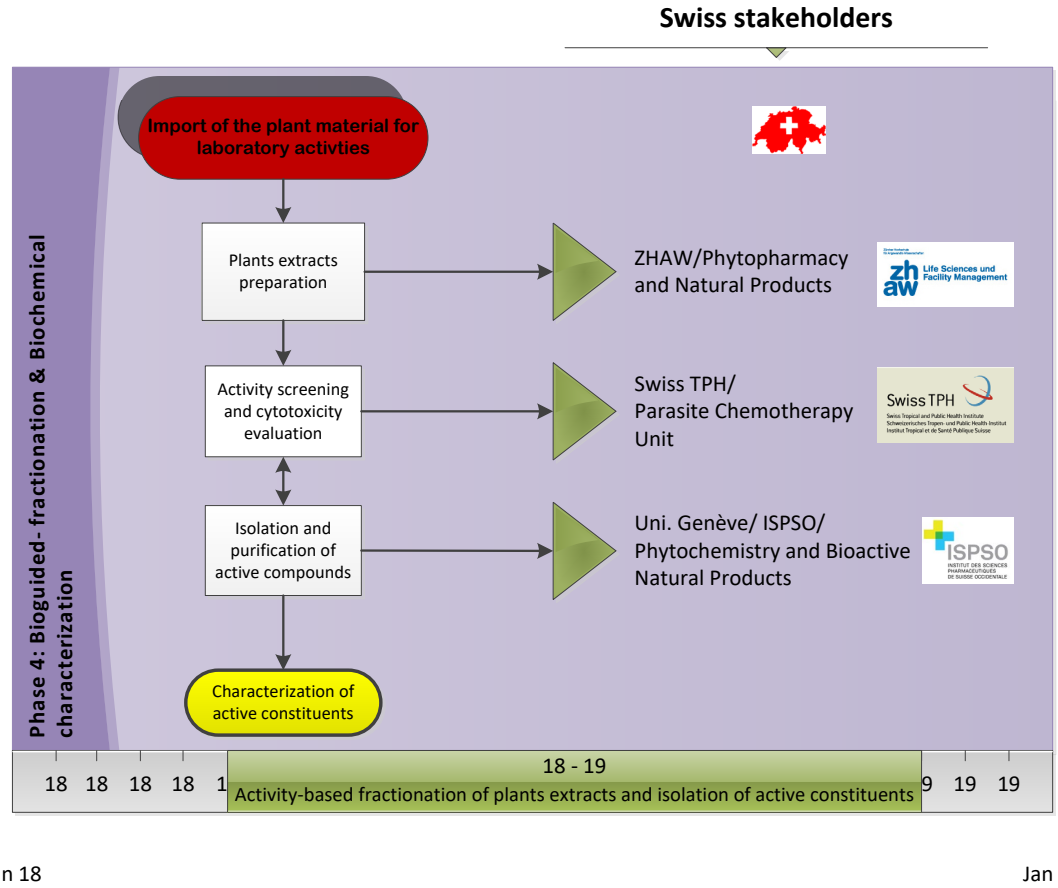


Figure 2-4: Phase 4 Bioguided-fractionation & Biochemical characterization.
After the import of the dried plant material to Switzerland, 3 scientific activities were carried out: (1) the preparation of the plants extracts in collaboration with the Phytopharmacy and Natural Products research group of the ZHAW, (2) the screening and cytotoxicity testing at the Swiss TPH in the Unit of Parasite Chemotherapy, (3) the isolation and purification of the active compounds in collaboration with the research group of the Phytochemistry and Bioactive Natural Products of the Swiss Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO) of University of Geneva. In green, the schedule bar indicates the duration of the scientific activities.

Phase 5: Feed-back session (January '20 – March '20)

Phase 5 will take place during the first trimester of 2020. It is the "closing phase" of the research project, dedicated to the dissemination of the results and to the promotion of communication and interaction between the various stakeholders. This last phase contributes to the third pillar of the *Convention of the Biological Diversity*, the sharing of the benefits. As in this case study the research did not generate any monetary benefit, phase 5 aims at sharing the non-monetary benefits, i.e. the gained knowledge. A workshop with academic and non-academic stakeholders will be organized as a round table, that will bring together all involved actors of the project to an interactive knowledge exchange. By taking into account the diverse perspectives of the project, this workshop aims at producing a new understanding of the benefits at stake and generating a co-produced knowledge (i) on the use of the reported botanicals in the management of sleeping sickness (ii) on the role of herbal medicine in the

primary health-care system. It will highlight the necessity to implement a validation process at national level.

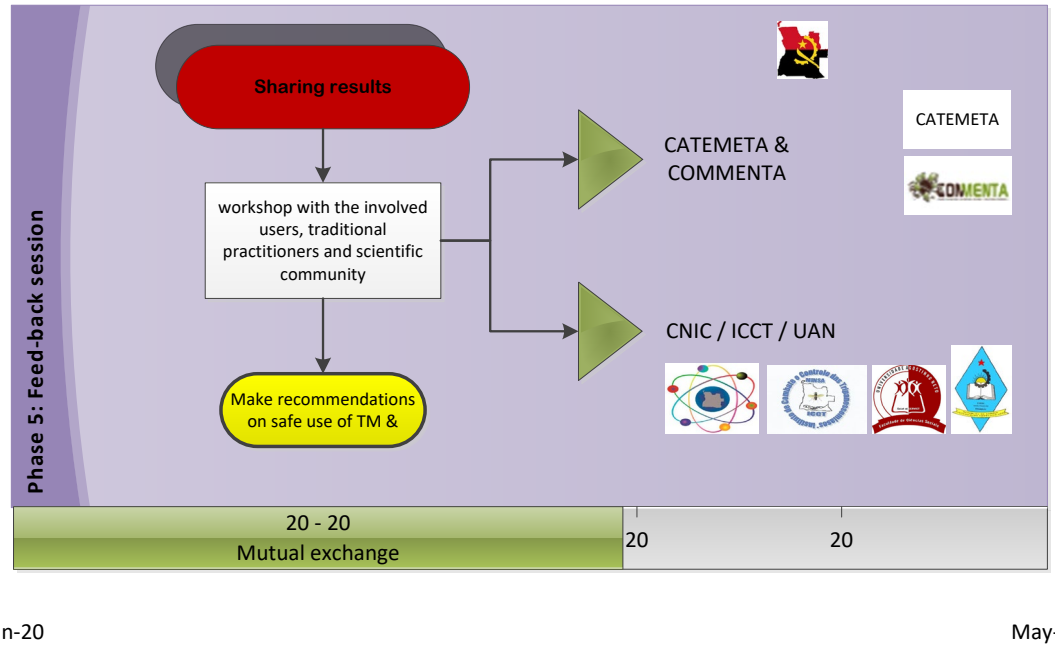


Figure 2-5: Phase 5 Feed-back session.
The result-sharing session will be realized in the form of a workshop with all involved Angolan partners. A mutual exchange of views and knowledge among the stakeholders will constitute the starting point of a co-produced knowledge on the use of Traditional Medicine (TM).

2.4 Results

By implementing and conducting research on medicinal plants in Angola and their scientific validation, three aspects in the procedural compliance had to be met, namely the Ethical aspect, Intellectual Property Rights, and Access and Benefit sharing. These three components and their legal framework were challenging due to:

- lack of an operational Ethics Committee;
- lack of binding measures relating to the IPRs of the traditional knowledge in hands of the traditional practitioners;
- lack of domestic ABS procedure.

The establishment of a scientific collaborative network from phase 1 to phase 3 has made it possible to meet the ethical, IPRs and ABS requirements.

Tables 2-1 to 2-3 present the different challenges encountered in these 3 domains, and the procedural adjustments that were realized so as to achieve at best compliance. The approval of the study protocol relates to ethical compliance, access to TK relates to IPRs compliance, and benefit sharing and access to GR relate to ABS compliance.

2.4.1 Ethical compliance

The study protocol had been approved by the Swiss Ethics Committee EKNZ (see Appendix 1, Doc 6). Nevertheless, in order to be fully compliant, the study protocol also needed approval from an Angolan Ethics Committee. The establishment of an inter-institutional collaboration between the CNIC and the ICCT bestowed a certain degree of legitimacy to the project in the Angolan research landscape. This alliance made it possible to create the conditions for an ethical approval, and a Ministerial Decree authorized the creation of an Ethics body *ad hoc*. Thus, in the absence of an operational Ethics Committee, an *ad hoc* Institutional Ethics Committee was created, with the support of the former Director of the ICCT (see Table 2-1).

Ethical compliance				
Target	Challenge	Collaborative Entity	Action	Output
Approval of study protocol	**No existing Ethics Committee**	ICCT	Creation of an institutional *ad hoc* Ethics Committee	- Ethical approval of the study from Angola - Authorization of the study implementation

Table 2-1: The ethical challenge regarding the compliance aspect. The targeted achievement (target), the local constraint impeding full compliance (challenge), the collaborative body that enabled a substitute mechanism (collaborative entity), the establishment of an adjusted measure (Action), the outcome of the measure (output).

As recommended in the *ICH E6 R2 Good Clinical Practice guideline* [63], the *ad hoc* Ethics Committee was constituted as follows: (i) it had to be made up of at least 5 members, (ii) at least one member did not have Science as primary area of interest, (iii) at least one member was independent of the ICCT. The PI had to submit the study protocol to the *ad hoc* Ethics Committee members and present the study design, its aim and objectives. After an open-questions session and closed discussion, the study protocol received approval (see Appendix, Doc 7) and the fieldwork was launched.

2.4.2 Intellectual Property Rights (IPRs) Compliance

Having obtained ethical approval from the Angolan part as well, the fieldwork started in phase 2 with the ethnobotanical study aiming at gathering information from traditional practitioners and patients on the local use of medicinal plants against sleeping sickness. Thus, accessing Traditional Knowledge associated with medicinal plants raised concerns about the intellectual property rights of the holders of this knowledge. At the time of this scientific phase, Angola was not yet party to the Nagoya Protocol. Consequently, from an international perspective, the intellectual property right measures included in the MAT were not applicable. Moreover, from a Swiss legal perspective, the Swiss regulation on Due Diligence did not apply when collaborating with a *CBD-only country*[18] [64]. Considering the Angolan legal context relating to the Traditional Knowledge and the Traditional Practitioners, the country had recognized within the *Law on Cultural Patrimony* in its Art.45/4 and 46/b that Traditional Knowledge associated to medicinal plants is a Cultural Good and it shall be protected by the State by different means; among others, by promoting protection of the Holders of the Traditional Knowledge [65]. In addition, by having signed the international *Convention on the protection*

[18] Countries that are party to the CBD but not yet to the NP are defined as "CBD-only countries"

and promotion of the diversity of cultural expressions, Angola has recognized that Traditional Knowledge is a cultural good, which is part of the diversity of the cultural expression and needs to be promoted as well as protected [66][19].

However, to the best of our knowledge, there are no regulatory measures in Angola that define how the Traditional Knowledge as a Cultural Good should be protected. In the absence of legal and administrative instruments, defining and protecting the status of the traditional practitioners as owners of the traditional knowledge, and applying Intellectual Property Rights over their knowledge, seems to be somewhat arbitrary compromised. In order to overcome this normative gap, we relied on the *Code of Ethics of the International Society of Ethnobiology*, which acknowledges in its first Principle that "local communities have prior, […] all knowledge, intellectual property and traditional resource rights associated with such resources and their use" [67].

From there on, three measures were taken to protect the IPRs of the traditional knowledge transmitted from the traditional practitioners to the research team (see Table 2-2).

IPR compliance				
Target	Challenge	Collaborative Entity	Action	Output
Access TK associated to medicinal plants	**No clearly defined status of the IP of the TK associated with medicinal plant**	Boards of the national traditional practitioners' association CATEMETA & CONMENTA	1. MOU	Transmission of TK associated to medicinal plants and protection of the IPRs
		Each interviewer	2. Confidentiality Agreement	
		INBAC	3. MAT/ Clause 8	Genetic Resources and associated TK is protected

Table 2-2: The Intellectual Property Rights (IPRs) challenge regarding the compliance aspect.

First, a Memorandum of Understanding (MOU) was signed between the Swiss TPH and each of the boards of the traditional practitioner associations, namely CATEMETA and CONMENTA (see Appendix 1, Document 2 and 3). In its clauses 8 and 10, the MOU integrated the intellectual property and confidentiality concerns. The assignment of the MOU should protect the traditional knowledge entrusted to the research team. Secondly, the interviewers signed an agreement to maintain due confidentiality related to any information obtained during the research period (see Appendix 1, Document 8). This agreement specified that any information protected by the intellectual property of a third party should be kept secret and could not be

[19] Angola has ratified the *United Nations Declaration on the Right of Indigenous Peoples* in 2007. In its Art. 31, it is pointed out that indigenous peoples have the right to "control, protect and develop their intellectual property over […] traditional knowledge. Moreover, the States shall "take effective measures to recognize and protect the exercise of these rights". However, according to the IWGIA, a global human rights organization dedicated to promoting, protecting and defending indigenous peoples' rights, "the Government of Angola does not recognize the concept of indigenous peoples as affirmed in international law, and there are no specific references to indigenous peoples or minorities in the Constitution, nor in other domestic law." Thus, a number of core human rights remain unrealized to the country's indigenous peoples (IWGIA website accessed on 24 November 2019: https://www.iwgia.org/en/angola).

divulgated. These two first agreements were established before Angola became party to the NP. Thereafter, the question of the Intellectual Property was addressed in the MAT, in order to protect the accessed genetic resources and associated Traditional knowledge. During phase 2 and phase 3 of the project, the question of the IPRs gave rise to three contextualized responses. These were elaborated in the form of an MOU, a Confidentiality Agreement, and a specific clause in the MAT to address the lack of a normative framework regarding IPRs of traditional knowledge associated to medicinal plants in the providing country. Besides this, the PI tried to raise awareness on this topic with training sessions to the involved students of the UAN. In addition, the challenges raised by the IPRs were addressed by the PI during the annual meeting of the CATEMETA, which was attended by a hundred traditional practitioners. The PI sensitized the traditional practitioners on the intellectual property rights they have on their own knowledge and practices.

2.4.3 ABS Compliance

One of the key intentions of the ABS procedure is to create a negotiation environment of trust and confidence among the stakeholders. The benefit-sharing principle was put in place in the frame of the academic collaborations during phase 2, which ran from October 2016 to March 2017. At that time Angola was not yet Party to the NP, thus there was no existing ABS mechanism. However, relying on the Code of Ethics of the ISE, specifically on the principle 12 "Reciprocity, Mutual benefit and Equitable Sharing", some ABS elements were defined and negotiated between the partners. Thus, the two academic collaborations with the department of Anthropology and the Center of Studies and Scientific Investigation on Botany enabled the integration of six bachelor students. five students in anthropology received training in scientific methodology, interviewing and data analysis. The PI supervised their field work and supported the bachelor theses. One student in botany received training in technical aspects of the field work and support in the elaboration of the master's work plan. Working material and instruments (as for example voice recorders or a GPS) were supplied by the Swiss Party. Consequently, before Angola was part to the NP, the benefit-sharing pillar was addressed with non-monetary benefits by providing training, capacity building and collaborative research (see Table 2-3).

Phase 3 started with the date Angola became Party to the NP (7th May 2017). Consequently, accessing the genetic resources of the providing country was subjected to the PIC and the MAT as defined by the Nagoya Protocol. The collection of the plant material required prior negotiation with the National Competent Authority (INBAC) and the National Focal Point in order to apply for the PIC. In a typical case, the PIC is applied by the *User* country before the research activities start. Once the PIC has been granted by the *Provider* country, the MAT will be elaborated. In our case, the scientific activities had been in progress since more than a year, and the research project had been made public, supported by the office of the Minister at the Ministry of Science and Technology. Therefore, applying for a PIC did not make sense. Consequently, to overcome the inherent contradiction in this procedural situation, a research project outline was submitted to the direction of the INBAC as well as to the National Focal Point, in order to inform on the project's aim and objectives, as well as on the accomplished scientific activities. Based on this document the National Competent Authority granted the PIC,

and it was decided to include the PIC formally in the MAT as a clause: " *The Provider hereby confirms that he has been informed on the research project by the User and consents to provide access to genetic resources in-situ and to carry out the research in accordance with the research project attached to this Agreement (Annex A)". The Provider herewith gives his Prior Informed Consent (PIC).*" (Clause 3 of the MAT agreement).

Phase 3: ABS Compliance				
Target	Challenge	Collaborative Entity	Action	Output
Benefit-sharing arrangements	**No domestic ABS procedure**	UAN, CNIC CATEMETA, CONMENTA, UAN, CNIC, ICCT	Cooperation with academic stakeholders Feed-back session (phase 5)	Scientific training, capacity building, collaborative research Results dissemination, co-produced knowledge on the use of TM
Access to GR and traditional associated knowledge	**Access to GR and TK prior ratification of Angola to NP (during phase 2)** **ABS procedure not in place, absence of domestic instrument to negotiate the MAT**	INBAC Swiss Academy of Sciences	PIC embedded in the MAT in Clause 3 Elaboration of MAT based on "ABS-agreement toolkit" as template	PIC formally granted by the national competent authority First MAT signed from Angola and Authorization granted to access GR

Table 2-3: The ABS challenge regarding the compliance aspect.

Given its recent membership to the Nagoya Protocol, Angola had not yet put in place a domestic ABS procedure, neither had it administrative procedures and documents to elaborate the MAT. Based on the "MAT toolkit" provided by the Swiss Academy of Sciences[20], a first draft of the *Mutually Agreed Terms* was established. After several negotiation sessions involving the National Competent Authority, the National Focal Point, and the PI, a definitive version was agreed upon in April 2018 (see Appendix 1 /Document 9). The agreement included monitoring measuresto guarantee compliance. Thus, all scientific activities and their results arising from phase 4 were submitted to the monitoring measure as defined in the MAT and reported biannually to the Angolan Party. According to the terms of the MAT, the two Swiss research partners (ZHAW and ISPSO) were considered as Third Parties accessing the genetic resource, thus subject to the initial conditions of the MAT.

[20] The "MAT-toolkit" is a booklet elaborated by the Swiss Academy of Sciences, which offers a toolbox to help set up the Mutually Agreed Terms. It contains Model Contractual Clauses and is designed for the use by providers of genetic resources and associated traditional knowledge and for academic researchers. The toolkit covers the essential elements that need to be considered in the case of access for academic research. The booklet is entitled "Agreement on Access and Benefit-sharing for Academic Research" and can be accessed at: https://sciencesnaturelles.ch/organisations/scnat/publications/swiss_academies_reports/79511-agreement-on-access-and-benefit-sharing-for-academic-research

The MAT that was granted in the framework of this collaboration in July 2018, was the first official MAT of Angola, as has been notified in the *Interim National Report on the Implementation of the Nagoya Protocol* published on May 2019 on the platform of the ABS-Clearing House[21]. According to Angolan ministerial procedure, the document had to be enacted by the Minister of Environment. Angola has yet to issue an "Internationally Recognized Certificate of Compliance" for its publication on the Access and Benefit-sharing Clearing House. In order to respect the international regulations regarding the trade of endangered species, the Angolan administrative authority of CITES delivered an exportation license, authorizing the exportation of "non-CITES" plant species to Switzerland (see Appendix/ Document 10). In addition, the INBAC, as competent organ of the Ministry of Environment, issued an export credential (see Appendix, Document 11).
To complete the established benefit-sharing elements, phase 5 will be dedicated to the exchange and sharing of the results and their benefits with the Angolan stakeholders, with particular emphasis on the traditional practitioners. By recognizing and valuing local knowledge on herbal remedies in the public health system, phase 5 will discuss the practical implications of the research project and the benefits at stakeholders' level. This closing phase aims to valorize the use of the herbal remedies on a scientific basis, and to raise awareness on the risk of use of certain medicinal plants. In this sense, phase 5 is a supportive element in the accomplishment of the benefit-sharing principle (see table 2-3).

2.5 Discussion

This case study questions the fulfillment of compliance requirements at national and international level in the framework of a transboundary project on the scientific validation of herbal remedies. More specifically, it refers to the ethical, IPRs and ABS requirements in the case of a scientific collaboration between a *User* country, which is party to the CBD and NP (Switzerland), and a Provider country (Angola) only party to the CBD at the time of the beginning of the collaboration, and which became party to the NP during the field research activities.

An important aspect identified was the necessity of a country to have domestic law and regulation systems in place that recognize the indigenous communities and their associated traditional knowledge. It is difficult for Traditional Knowledge to be protected by conventional intellectual property protection systems. Indeed, the conditions for patentability (novelty, inventive step, and industrial application) are difficult to reconcile with the nature of Traditional Knowledge. Though an adequate argumentation of this point is beyond the scope of this case study, a possible way to solve the question of IPRs of traditional knowledge and to protect the traditional practitioners could be by the elaboration and development of *sui generis* initiatives at regional and national levels [68]. Some African countries like the United Republic of Tanzania, Botswana, Burundi, Egypt, Kenya, South-Africa have adopted *sui generis* systems

[21] https://absch.cbd.int/countries/AO

in order to protect their Traditional Knowledge and promote its medicinal practice[22]. Mali, for example, adopted in 1995 with the Decree N°95-009/P-RM an enforcement of the Intellectual property legislation to regulate the registration and marketing authorization of traditional medicine [69]. At international level, the World Intellectual Property Organization (WIPO) established in 2000 a special body, the Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore (IGC), addressing the question of intellectual property and traditional knowledge [70]. Beside supporting the development of *sui generis* initiatives as a contextualized response at regional or national level, the IGC advocates the establishment of registries and databases of the traditional knowledge [71, 72]. These tools will enable to formally recognize the traditional knowledge and consequently protect it.

A state should define and recognize its indigenous groups or local communities and thereby clarify their legal status and the issue of their representativeness. Besides the need for effective measures for protection of TK, there is a need for awareness among the local communities, traditional practitioners, scientific communities, and government bodies about the intellectual property rights associated to the use of traditional herbal remedies. In this context, the role of the researcher from the *User* country is to contribute to the awareness-raising about the IPRs related to the traditional knowledge among the involved community. This will not only strengthen a trust relationship between the parties but also ensure greater respect for ethical aspects and the Intellectual Property Rights. At best, it can favor an endogenous empowerment of the holders of the traditional knowledge and encourage them to claim for their rights to be protected by the state.

Concerning the ABS requirements, the conceptual lack in the CBD and NP about the indigenous and local communities may lead to practical difficulties in defining the proper recipients of the benefit, particularly if the *Provider* country has not yet put in place policy and legal measures to recognize the indigenous and local communities. As was experienced in this case study, none of the ABS measures included during this ethnopharmacological research had been directly negotiated with the holders of the traditional knowledge. Main difficulty was the definition of who was the recipient of the benefits. Indeed, the traditional practitioner's group was a heterogeneous group composed of traditional practitioners dispersed across two provinces in Angola, belonging to two distinct ethnolinguistic groups. However, they all belonged to one of the two main traditional practitioner associations (CATEMETA and CONMENTA). Therefore, a MOU covering the question of the IPRs was elaborated directly with the board of these two associations. However, the MOU did not specify any benefit-sharing eleements, because the stakes of power within the associations could have compromised an equitable distribution of the benefits. Concerning the MAT, it was negotiated when phase 2 activities were already finalized, therefore it did not include a clause addressing the sharing of the benefits with involved communities and traditional practitioners.

[22] See World Intellectual Property Organization database of *sui generis* laws: http://www.wipo.int/tk/en/databases/tklaws/.

Under these circumstances, phase 5 has to be considered not only as a feedback session of the results but especially as an adjustment measure regarding the benefit-sharing requirements toward the involved community and the traditional practitioners. Here again, the role of the scientific partner from the *User* country is crucial to negotiate and propose alternative compensating measures to benefit the involved holders of the traditional knowledge. As example, the return session in phase 5 is an initiative from the PI, sponsored by the Swiss party. The unclarified responsibility regarding the application of the ABS measures at institutional level in Angola[23] illustrates the complexity of transcribing and adapting international treaties into domestic regulations [73]. The role of international collaborations in triggering and accelerating the adjustment of national administrative, policy and law measures to these international treaties must be underlined. Indeed, through this collaborative research project between Switzerland and Angola, and more specifically between the Swiss TPH and the CNIC, competent authority (INBAC) issued the first MAT in Angola, even though no formal ABS regulations were in place at national level.

From a Swiss legal perspective, accessing genetic resources in a country that is party to the NP, even though lacking ABS regulation, requires to meet "due diligence" as defined in the Swiss Ordinance on *Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization*. In its Art. 3, the due diligence requirement asks the *User* of the genetic resources to "*record, keep and pass* […] *to subsequent users* (a) *the internationally recognized certificate of compliance issued in accordance with the provisions of the Nagoya Protocol* […]. If any, the Article 3 provides a list of detailed information on the accessed GR and its user. In that sense, the procedural ABS requirements of this research project fulfilled the Swiss legislation, with all detailed information included in the MAT and its annexed documents.

According to the monitoring measure in the MAT in its Clause 14, the *User* should transmit a biannual report on the scientific activities and results arising from the use of the genetic resources. This measure was fulfilled accordingly along the research phase 4. However, no feedback was received from the Angolan party. This might be due to the difficulty to follow the research process for non-expert persons involved in the MAT agreement. The traceability of the research progress is surely an essential tool to prevent misunderstanding and disruption of trust. In that sense, non-technical communication of the research results contributes to ensure compliance with the agreed terms under the MAT. However, if traceability is to support the procedural fairness, there is a need for concerned personnel of both, the *Provider and User* country, with sound knowledge of the scientific part and cultural context of the research project to be agreed upon. As so, an effective follow-up of the scientific activities and a fruitful exchange will be implemented as the promise of a fair and informed interaction between the parties.

This case study demonstrates that scientific validation of traditional herbal remedies is a process that requires the competence of several field of expertise and is therefore, meant to be transdisciplinary work. In the absence of adequate laboratory equipment and expertise,

[23] See the *Interim National Report on the Implementation of the Nagoya Protocol* published on May 2019 on the platform of the ABS-Clearing House, https://absch.cbd.int/countries/AO

notably in phytochemistry, as is the case in Angola, developing collaborations is essential to implement such a scientific validation process. In this highly collaborative environment, compliance measures are crucial to develop a trustful negotiation environment and to establish confidence among the parties involved.

Considering our general research question, we conclude that compliance will be achieved based on four key elements: (i) a strong network among the different stakeholders of the project, (ii) sound knowledge, (iii) interest in both the *Provider* and the *User* country in understanding the scientific challenges and the compliance requirements, (iv) a high standard of legal and ethical conduct in all the scientific dealings.

2.6 Conclusion

The main lesson to be learned from this experience lies in the importance of developing and strengthening local networking when implementing a transboundary research project. Locally based-collaborations will reinforce the project at local and regional level as well as building trust among the parties. However, the respect and application of a good scientific practice, by following the ethics codes and doubled by a sense of accountability, can achieve compliance so far as the legal responsibility of the partner country starts.

We recommend that transparency, fairness, and mindfulness serve as guiding principles whenever the legal framework is insufficient to regulate scientific decisions about cooperation.

3 USE OF HERBAL REMEDIES IN THE MANAGEMENT OF SLEEPING SICKNESS IN FOUR NORTHERN PROVINCES OF ANGOLA¶

Nina Vahekeni[a,b,c], Pedro Menezes Neto[d], Malilo Kaluiji Kayimbo[c], Pascal Mäser[a,b], Josenando Théophile[e], Esperança da Costa[d], Jacques Falquet[f*], Peter van Eeuwijk[a,b,g*]

[a]Swiss Tropical and Public Health Institute (Swiss TPH), Socinstr. 53, 4051 Basel, Switzerland

[b]University of Basel, Petersplatz 1, 4001 Basel, Switzerland

[c]Nacional Center of Scientific Investigation (CNIC), Luanda, Angola

[d]Centro de Estudos e Investigação Científica de Botânica, Universidade Agostinho Neto, Luanda, Angola

[e]Instituto de Combate e Controlo das Tripanossomíasses (ICCT), Luanda, Angola

[f]Community Health Association, Geneva, Switzerland

[g]Institute of Social Anthropology, University of Basel, Münsterplatz 19, 4051 Basel, Switzerland

* JF and PvE share responsibility in equal parts

2020

Journal of Ethnopharmacology, 256, 112382

I conceived the study design and wrote the manuscript. The interviews with the traditional practitioners were performed by myself. Some of the interviews with the patients were conducted by students under my supervision.

3.1 Abstract

Ethnopharmacological relevance: This study reports for the first time on the use of folk medicine to treat sleeping sickness and its symptoms in four endemic provinces in northern Angola. By interviewing both traditional practitioners and confirmed patients, it highlights reasons to recourse to folk medicine, the plant species used for this affection as well as arises awareness about the use of particular plants showing potential risks.

Aim of the study: The aims of this explorative study were three-fold. Firstly, it informed on access to, and use of plant-based medicine as first-choice treatment by infected persons. Secondly, it aimed at collecting comprehensive data from patients and traditional healers on herbal remedies in order to identify plant species used in the management of the disease. Thirdly, it served as contribution for primary indication of potential risk of use associated with the studied plants and their preparation.

Materials and Methods: The study was conducted in 4 endemic provinces of Angola, namely Bengo, Zaire, Kwanza Norte and Uíge. We explored the use of herbal remedies by conducting structured and semi-structured interviews within two distinct study populations. The first group comprises 30 patients who had been diagnosed for trypanosomiasis and treated by the reference treatment. The second group included 9 traditional practitioners who had already treated sleeping sickness. The plants that were cited during the interviews were collected during field walks under supervision of a traditional healer, then authenticated and deposited at the National Herbarium in Luanda.

Results: Of the 30 included patients, 12 (40%) had turned to folk medicine in the management of trypanosomiasis and related symptoms. 7 medicinal plants were reported by this group. Considering the key motivation to consult a traditional practitioner, two main factors accounted for half of the cases: “past experience with folk medicine” and “family habit”. Out of 9 traditional practitioners’ interviewees, 26 medicinal plants were cited. Roots and leaves were the most used plant parts, and decoction was the common mode of preparation. Evidence for antitrypanosomal activity in the scientific literature was found for 56% (17 of 30) of the identified plant species. The most cited plant was *Crossopteryx febrifuga* (UR=6). Some of the cited plants, as for example *Aristolochia gigantea*, raised concern about potential toxicity.

Conclusions: With 40% of infected persons having turned first to folk medicine before consulting a medical doctor, this explorative study points out that plant-based medicines play an important role in local dynamics of health care. It highlights the need for primary assessment of potential risk of use related to the herbal recipes, and for reporting it to the concerned population. This first ethnobotanical study on trypanosomiasis in endemic provinces of Angola provides information on 30 plants, of which some had been identified as promising for further pharmacological research. Our results provide a first step towards the validation and valorization of Angolan herbal remedies for sleeping sickness.

3.2 Introduction

The extensive use of folk medicine (FM) in Sub-Saharan Africa, composed mainly of medicinal plants, has been argued to be linked to cultural and economic reasons. The accessibility and availability of qualified physicians is limited, with counts of only one medical doctor for 40'000 persons [20]. This is why WHO encourages African member states to promote and integrate validated traditional practices in their health system [23]. In Angola, more than seventy percent[24] of the population uses herbal medicine to treat various medical affections, including parasitic infections.

Human African Trypanosomiasis (HAT) is a protozoan Neglected Tropical Disease (NTD) transmitted by tsetse flies (*Glossina* spp.). It is caused by two different subspecies of *Trypanosoma brucei*: *T. b. gambiense* causes chronic infection and is prevalent in west and central Africa, while *T. b. rhodensiense* causes a more acute infection in eastern Africa. Both forms of the disease are fatal if untreated. At the time of the study, the reported number of new cases in Angola was 35 for 2014 and 34 for 2015. The standard medical treatment uses five different drugs, depending on the disease stage: pentamidine and suramin are used in the first, hemolymphatic stage; melarsoprol and nifurtimox-eflornithine combination therapy (NECT) in the second stage, the cerebral stage [2, 74]. Fexinidazole is a new drug, the first-ever oral treatment for all stages of *T. b. gambiense* HAT that successfully passed clinical trials and was recently endorsed by the European Medicines Agency [6, 75]. This new and easy-to-apply drug for both stages of the disease constitutes a huge leap forward in the treatment of sleeping sickness and is a step forward to the elimination of HAT. Acoziborole is an additional oral treatment in the development phase meant to be given as a single dose treatment for both stages [76]. All drugs are donated by the manufacturers, and WHO guarantees free-of-charge distribution [1]. There is no vaccine against trypanosome infection, and chemoprophylaxis is not used because of the toxicity of the available drugs and the low incidence of infection.

The geographical distribution of HAT is highly contingent upon environmental conditions for the *Glossina* flies. Sleeping sickness is found in the northwestern part of Angola and is prevalent in seven provinces out of eighteen [14]. Bengo, Kwanza Norte, Uíge and Zaire are the four highest risk areas and 5.8 million people are at risk [13]. We know that the disease mainly affects remote rural communities, where health infrastructure is basic and its accessibility complicated [7]. Moreover, the number of reported cases relies on systematic control activities and national surveillance program. The current economic recession that impacted the stability of the national economy in Angola since 2015, affected the efficiency of screening activities [private communication/ medical team of ICCT, 2016]. As a consequence, a certain number of cases are undetected and untreated by the surveillance programs, leading to a gap between the number of cases declared and the number of actual cases [14] . In absence of vaccine and chemoprophylaxis, the infection is mainly controlled through case detection and treatment. Thus, HAT can upsurge, if control measures should be relaxed, for example in the context of conflict or socio-political instability [10].

[24]Percentage given at the 1st *National Conference of Traditional Medicine and Complementary Practices* held in Luanda in August 2012.

In such context, the investigation of herbal remedies as a natural affordable and accessible resource is of high relevance. The scientific validation of an herbal preparation, through its botanical and pharmacological assessment, guarantees a safe and effective use of the remedy and contributes in that sense to strengthening of the health system. Thus, for hard-to reach communities much exposed to the disease with difficult access to healthcare, the use of a "validated" recipe in the management of sleeping sickness would be a complementary response for the improvement of health conditions and a mean of valorizing local medicinal knowledge.
This prompts us to investigate whether or not folk medicine is used to treat sleeping sickness, and to assess the potential use of herbal medicine in case of infection by this parasitic disease. Therefore, the following research questions were addressed: (i) among the infected and treated persons, how many had turned to FM before consulting a medical doctor? (ii) what are the therapeutic options followed by the patients infected with trypanosomiasis? (iii) what are the botanical species used in the management of sleeping sickness? (iv) are there any potential risks of use associated with the studied plant species?

The last decades have witnessed a range of investigations reporting antitrypanosomal activity of traditionally used African medicinal plants, and several reviews compiling the major results have been published [47, 48, 77-83]. Concerning Angola, a thorough description of the use of medicinal plants in central and western parts of Angola was provided by Eric Bossard [84]. A few ethnobotanical studies described the knowledge of traditional use of plants in Angola [85-92]. Da Costa and Pedro [93] summarized in their book general ethnomedical information about medicinal plants commonly used in this country. All the above mentioned works focused on traditional knowledge. Apart from a recent ethnopharmacological study on anti-inflammatory effect of medicinal plants from Angola [94], there has not been a previous ethnopharmacological study reporting the use of herbal remedies to treat a parasitic disease in Angola.Due to the paucity of ethnopharmacological scientific data on HAT in Angola, we conducted an explorative study among infected and already treated persons (patients) as well as among traditional practitioners in order to gather valuable primary information about the use of herbal medicine as first-line treatment for HAT.
Our ethnopharmacological explorative study aims at (i) reporting the use of folk medicine to treat sleeping sickness and related symptoms in endemic provinces of Angola, (ii) documenting the used medicinal plant species (iii) identifying potential risks related to certain plant species and their preparation.

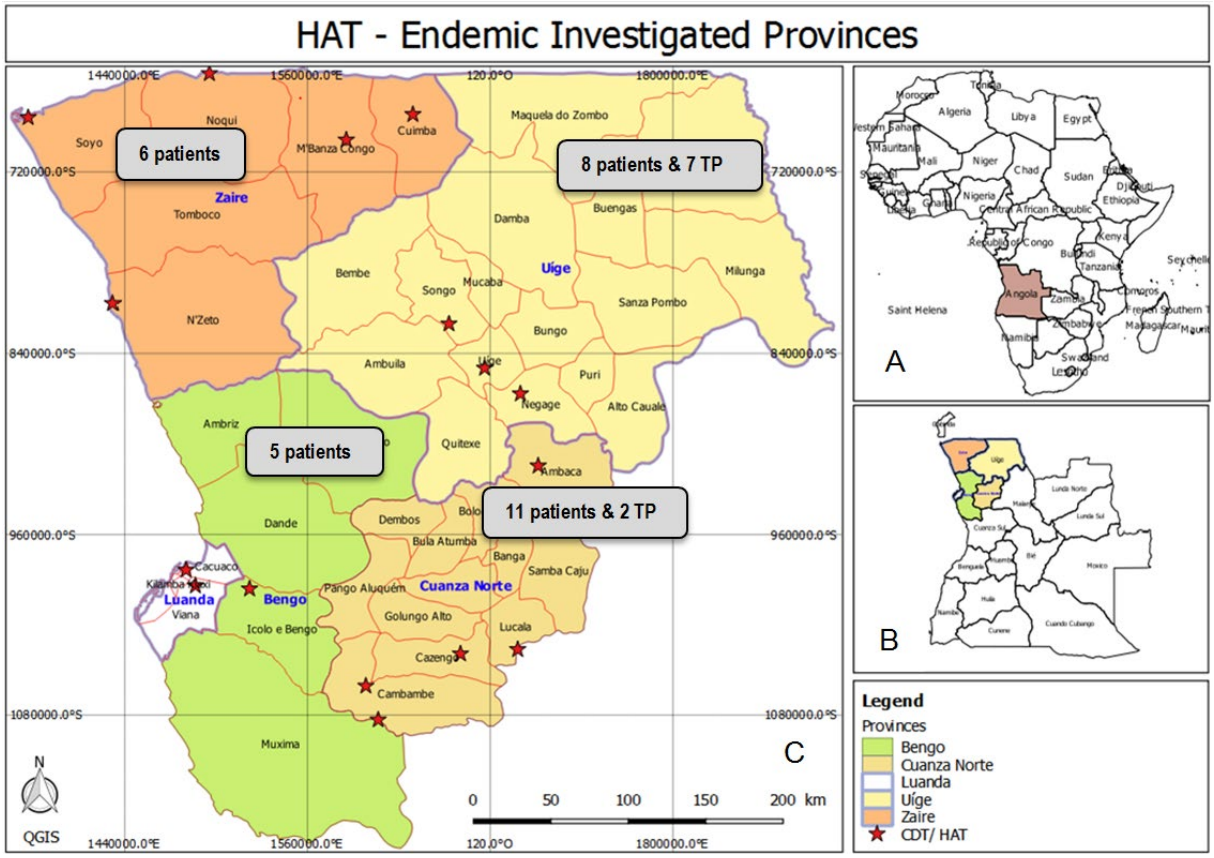
3.3 Materials and methods

Study area

Angola counts 18 provinces, 162 municipalities and 559 communes. This explorative study took place in the four highest-risk provinces of Angola, namely Bengo (green), Kwanza Norte (mustard), Uíge (yellow) and Zaire (orange)(fig.3-1). Bengo (highlighted in green) and Kwanza norte (highlighted in mustard) are the two least populated provinces of Angola. Two ethnolinguistic groups can be found among these four provinces. The first goup, namely

Quicongo (Kikongo ou Conguês) is composed of Bakongo people and covers the provinces of Zaire and Uíge and a part of Kwanza-Norte. They mainly speak kikongo, one of the national language. The second ethnolinguistic group is represented by the Quimbundo (Kimbundu ou Tyumbundu). It is composed of the Mbundu people and includes the provinces of Bengo and part of Kwanza-Norte. Their language is the kimbundu, another national language.

Figure 3-1: Geographical location of Angola in Africa and the four studied provinces in colors.



Legend: CDT/HAT- Trypanosomiasis Diagnostic and Treatment Center

A: Angola is located in the southern part of Africa. B: the four northern endemic provinces that were inquired. C: Investigated provinces and the number of respondents per provinces. Province of Zaire (orange)/ 6 patients were interviewed; province of Uíge (yellow) / 8 patients interviewed and 7 traditional practitioners (TP); province of Bengo (green)/ 5 patients interviewed/ province of Cuanza Norte (mustard)/ 11 patients interviewed and 2 traditional practitioners (TP).

Municipalities covered by interviews of patients and traditional practitioners were: Dande, Ambriz for Bengo province; M'Banza Kongo for Zaire province; Uíge, Songo, Makela do Zombo, Beu, Bungo for Uíge province; and Cazengo, Banga, Dondo for Kwanza Norte province. Figure 1 shows the number of participants interviewed per province.

At the time of the study, 15 fixed health centers were responsible for diagnostics and treatment of HAT in the 4 inquired provinces (location highlighted by a red star/ fig. 3-1), covering approximately 150 000 km^2 and a total population of 2'877'573. The detection of new cases relies on active screening by mobile teams and passive screening in the fixed health centers. In 2014, Angola officially registered 36 new cases, followed by 35 in 2015 [95] and 18 in 2017.

Except in the urban and peri-urban areas, the surveyed area is mostly rural, characterized by activities like farming, hunting and fishing. The rural environment offers suitable conditions for the development of tsetse flies and human-tsetse contact. The traditional practitioners

mainly originated from Uíge province. This reflects a strong presence of FOMETRA in that province, one of the two most important organizations of traditional practitioners at national level. In Uíge FOMETRA counts 14'201 traditional practitioner members. In comparison, Bengo counts 2703 traditional practitioners, Zaire 1744 and Cuanza Norte 1936 (approval by [Avô] Kitoko Maiavanga, coordinator of CATEMETA, personal communication, 2017).

Study population

There were two distinct study populations, patients and traditional practitioners. The patients' population consisted of participants treated for trypanosomiasis within 2014 and 2015 by the *Instituto de Combate e Controlo das Tripanossomíases* (ICCT) and its provincial medical centers. Out of 60 patients, 30 were interviewed, the remaining half consisting of patients who could not be reached or had to be excluded because they were less than 18 years old. The enclosed patients were under follow-up treatment, which allowed us to work with clinically confirmed and treated cases, a crucial point for medical and ethical reasons. Access to medical records helped us to geolocate the participants. This design enabled us to investigate whether the patients had turned first to folk medicine, before consulting a medical doctor, and by consequence to assess the use of herbal medicine for clinically confirmed cases.

The specialists' population was composed of 9 traditional practitioners originating either from Uíge Province or Kwanza Norte. They are members of *FOMETRA* or *CONMENTA*, the two most important national healers' associations. The traditional practitioners were selected first on recommendation of the scientific leader of FOMETRA and the correspondent provincial director, based on their reputation in the treatment of sleeping sickness. Independent advice was obtained from the municipal representative of the association, who, as a local contact, was foremost aware of the competence of the traditional practitioners regarding HAT. The selection was also influenced by the willingness of traditional practitioners to participate in the study. Finally, only 9 traditional practitioners have fulfilled the inclusion criteria (see supplementary file S1, study protocol).

Data Collection

Data were collected over a 5-month period, from October 2016 to March 2017, covering about 25 different localities distributed in 4 provinces. The interviews were led in Portuguese and, when needed, the questions were translated orally to local dialect (Kikongo or Kimbundu) by a field assistant that was either a member of the group or a translator. Two different questionnaires were used (see supplementary data S2 and S3), both of which included general sociodemographic features like age, sex, location, level of education and spoken language. Local name of disease, symptoms of disease, cause of disease and disease transmission were also asked to both groups of respondents.

The interview sessions with traditional practitioners were realized in the privacy of their home and lasted for an average of one hour. The questionnaire involved a set of questions including category of healer, local name of plant, mode of preparation, mode of administration, precaution of use, focusing on the herbal treatments used as well as their representation and the healer's understanding of sleeping sickness. A second session was organized to collect the

plant material during field walks under guidance of the respondent or of the local representative traditional practitioner.
Also the patients' inquiries were conducted at their home and included questions about distance to next health center, type of first choice treatment, key motivation to use FM, frequency of use of FM. Some of the mentioned plants were also collected during field walks.
Before each interview voluntary participation was previously confirmed by a written informed consent.
Ethical approval for the study (study protocol and the informed consent form) was obtained by the Swiss *Ethikkommission Nordwest- und Zentralschweiz* (EKNZ BASEC Req-2016_00403) and the Angolan *Comité da Ética ad hoc* (Ministerial decree of 14.09.2016, ofício N°2469/ GAB.MIN/MS/2016).

Plant Material
The plant specimen described by specialists and/or by patients were collected during field walks in Uíge and Kwanza Norte provinces (highlighted in yellow & mustard/ fig.3-1). Plant sampling was carried out in provinces of Uíge and Kwanza Norte. The collecting area extends from 6°01.173' and 9°19.867' southern latitude and 14°49.401' and 25°23.914' longitude. The main part of sampling took place in municipalities of Uíge, Maquela do Zombo, Damba of Uíge province and Kazengo and Kambambe of Kwanza Norte province.
The plants were collected based on the vernacular name cited by the informant. The herbarium specimen was photographed and collected during field work, carefully pressed and dried, and stored at the National Botanical Center in Luanda, which is part of the Faculty of Sciences at University of Agostinho Neto. A first determination was done with assistance of specialized botanists. Authentication was then carried out at the National Botanical Center in Luanda. For identification of plants, we determined the family, genus and species by (i) comparing with existing herbarium specimens (ii) consulting the Herbarium database of the National Botanical Center and specialized databases, including *Tropicos*, the *African Plant Database*, *The Plant List,* (iii) by referring to floristic references [96, 97].

Data analysis
Qualitative data were analyzed with MAXQDA software. Fisher's exact test and the Wilcoxon rank sum test were used to compare categorical and numerical variables, respectively, between the FM- and PM-patients. Collected plant data were treated with open-source QuantumGIS 2.14.8-Essen software. To quantify the collected ethnobotanical data, we recorded the use report (UR). Every plant use reported in a herbal recipe was considered as a separate record and counted as one use report[98]. If a plant species was mentioned several times in different recipes, the use report was accordingly counted.
The online bibliographic databases *Pubmed*, *Science direct*, and *Google scholar* were consulted for studies on antitrypanosomal activity of the identified plant species. The literature search was conducted by using the botanical name (and its synonyms) of the studied species in combination (Boolean AND) with the following keywords: sleeping sickness, trypanosomiasis, antitrypanosomal activity, folk use, traditional use, ethnopharmacological use, ethnomedical use, and ethnobotanical use. Further articles were included by tracking the cited references.

Studies that explicitly reported on plant extract tested for in vitro and/or in vivo antitrypanosomal activity were selected.
In order to estimate potential risks of toxicity for each species, online bibliographic databases such as *Pubmed* and *Science direct* were consulted.

3.4 Results

3.4.1 The use of folk medicine in the case of sleeping sickness

3.4.1.1 Local nosology

Sleeping sickness is known in Portuguese, the official language in Angola, as "doença do sono" and is frequently referred to by a local name according to the spoken ethnic language. In the four inquired provinces, two main national languages are used, namely Kimbundu and Kikongo as well as dialects that are particular to a region. As such, sleeping sickness has different appellations for example the term "Manimba", literally translated as "sleep", is used in dialect of Kikongo spoken in the province of Uíge or "Tonji", translated to "sleep", is a local appellation used in Kimbundu dialect spoken in the province of Bengo. These different designations of the illness in local tongues refer to the most characteristic symptom of the disease which is sleepiness.

3.4.1.2 The users of folk medicine

The selected 30 patients had all been diagnosed with T. b. gambiense HAT (g-HAT) and received modern chemotherapeutic treatment. 12 (40%) of them admitted to having previously received traditional remedies. Thus, two distinct groups resulted from the inquiry: the folk medicine (FM) patients group (n=12) who had first consulted a traditional practitioner, and the professional medicine (PM) patients group (n=18) who had directly turned to a biomedical doctor (see Table 3-1).

Table 3-1: Number of patients with sleeping sickness according to first choice of treatment

Type of first treatment	Provinces inquired				Total number of patients	Percentage (%)	Total percentage (%)
	Bengo	Zaire	Kwanza norte	Uíge			
Biomedical reference treatment (active[a])	1	0	2	1	**18**	13.3%	60.0%
Biomedical reference treatment (passive[a])	1	4	6	3		46.7%	
Herbal treatment from traditional healer/herbalist ("curandeiro")	1	1	2	3	**12**	23.3%	**40.0%**
Herbal treatment from sorcerer ("kimbandeiro")	0	1	0	1		6.7%	
Herbal self-treatment ("caseiro")	1	0	1	0		6.7%	
Other[b]	1	0	0	0		3.3%	
Total	5	6	11	8	**30**	100.0%	100.0%

The patients are distributed according to their first treatment choice and the province they originate.
Legend: [a] type of screening; [b] herbal treatment given by a Chinese medical center located in Luanda

In more than half of the cases (7 out of 12), the herbal treatment was provided by a traditional healer ("curandeiro"), whereas two patients consulted a sorcerer ("kimbandeiro") and two

others used a self-herbal treatment ("caseiro"). In one case, the herbal remedy was provided by a Chinese doctor from a Chinese medical center.

When comparing the distribution of socio-demographic and health-care related determinants (age, gender, education level, primary occupation, distance to next health center, previous experience with folk medicine and economic level) between the two groups of patients, we found no sizable differences according to age, gender or household economic level. Among all the determinants studied, previous experience with folk medicine was the only one that significantly impacted the recourse of herbal remedies in the management of sleeping sickness: 11 patients (of 12) of FM patients group had already used folk medicine (p= 0.002) compared to 6 patients (of 18) of the PM group. The symptomatology and the family habit were two key motivations that patients evocated for consulting a traditional practitioner. Concerning the symptoms, severe and long-lasting neuropsychiatric disorders like behavioral change and somnolence were mentioned as characteristic signs of illness that prompt the patients to turn to a traditional practitioner. Added to that, the influence of family habit in health-seeking behavior played an important role in the therapeutic option. Concerning the 12 patients' satisfaction with the herbal treatment, 3 were satisfied mentioning that "*it helped"* or *"it relieved*, but it didn't cure. The remaining 9 patients were not satisfied reporting that the herbal preparation had no effect or that traditional practices were not trustful based either on disappointing experience or negative perception of the traditional practitioner. The patients provided two main explanations for the cause of the disease, either a supernatural origin or a pathogenic agent. No significant difference related to local etiology was observed between the FM group and the PM group.

3.4.1.3 The providers of folk medicine

Even though there are 80 times more traditional healers than doctors in Angola, only few traditional practitioners treat it. We had access to 9 practitioners known for having already treated sleeping sickness, none of which claimed to be a specialist for this indication. The most experienced traditional practitioner, whose experience referred to epidemic periods, had treated more than 20 patients, whereas the others had treated only a few cases. The main signs on which they rely to identify the disease are sleepiness, behavioral change and a blurred vision. All these clinical features evocated by the traditional practitioners indicate mainly symptoms of the second phase of the disease. On the contrary, first phase symptoms are unspecific, as for example, chronic or intermittent fever, headache, itching and lymphadenopathy. In contrary to the patients, all traditional practitioners had a particular idea about the illness etiology and they mainly attributed sleeping sickness to the bite of a fly or a mosquito or even spiritual influences. They generally don't know that it is lethal when untreated. Finally, in case of treatment failure, they mostly either changed their herbal prescriptions or recommended another specialist. In two cases, the patient could optionally be redirected to a health center. One spiritualist attributed treatment failure to sorcery intervention.

3.4.2 The variety of plants used in the treatment of trypanosomiasis

A total of 37 plants was recorded of which 30 could be identified (see Table 3-3); 7 were presently not followed up because of lack of complete specimen .The plants name has been checked with http://www.theplantlist.org accessed on 1st of June 2019.

Of the 30 identified plants, 23 were only mentioned by the traditional practitioners, 4 by patients and 3 by both groups (see Table 3-2 /see column "informant group" - Sp & nsp). 6 patients of the FM group, could name the plants in the herbal mixture used. As evidence, the two patients that had referred to self-medication, could easily name (vernacular name) and describe the plants used in the herbal remedy. 26 plants were mentioned by 9 traditional practitioners, corresponding to 9 different treatments made of 23 different herbal recipes. Each recipe specifically targets a symptom. The herbal treatments were made of one to three recipes. Most recipes (16 of 23) were made of 1 plant, and only two recipes were composed of 5 different plants. Table 3-2 shows an example of herbal treatment made of 3 herbal recipes.

Table 3-2: Example of an herbal treatment made of 3 different recipes

Recipe 1: nasal drops	**Recipe 2: drink**	**Recipe 3: drink**
Entada abyssinica / R	Crossopteryx febrifuga / L	Sarcocephalus latifolius/ R
	Vitex madiensis /L	Momordica charantia / R
		Fleroya stipulosa / T
		Crossopteryx febrifuga / R
Mode of preparation. Direct use of crushed fresh root **Mode of administration:** nasal droops **Use:** alleviate headache	**Mode of preparation:** decoction **Mode of administration:** drink **Use:** against somnolence & eyes trouble	**Mode of preparation:** decoction **Mode of administration:** drink **Use:** "antibiotic"

Plant parts used: R: root, L: leaves, T: trunk

The most cited mode of preparation was decoction in boiling water for about 15 minutes. Less frequently mentioned were macerations, infusions and crushed fresh plant material for direct application or intake, as for example cranial cataplasm or ophthalmic drops. The most represented family was the *Rubiaceae* with four different species mentioned, out of which *Crossopteryx febrifuga* was the most cited plant; it was included in 6 different recipes and was used by specialists as well as by patients. *Vitex madiensis*, *Momordica charantia* and *Palisota schweinfurthii* were equally cited (UR=3, see Table 2) exclusively from specialists. Leaves and underground organs (root, rhizome) were the most commonly used plant parts. Except for one traditional practitioner, who claimed that *Trilepisium madagascariense* and *Daniellia alsteeniana* were specific for the treatment of HAT, all other specialists mentioned at least one other indication the plant was used for, like for example malaria, diabetes, convulsions or infections. None of the studied plants is endemic and three species are naturalized (see Table 2, indicated by double asterisk**). 4 plants species, *Azadirachta indica, Bryophyllum pinnatum, Daniellia alsteeniana,* and *Gardenia ternifolia*, were claimed to be used as prevention either as a repellent by rubbing crushed fresh leaves on the skin or in the form of oral intake as a fortifying drink. In terms of treatment, only one traditional practitioner differentiated the plants to be used according to disease stage: *Steganotaenia araliacea* was claimed to be used specifically in early phase against fever, headache, and great tiredness whereas *Palisota schweinfurthii* in advanced phase against behavioral change and somnolence.

Table 3-3: Plants used by patients or/and traditional practitioners in the management of trypanosomiasis

*Local name are systematically given in Kikongo; when another language is used, it referred either to Kim: Kimbundu or Pt: Portuguese. ** naturalized plants species.
Legend: Wp: whole plant, L: leaves, R: roots, T: trunk, Ap: aerial part, Tb: trunk bark, Rb: root bark, S: seeds, Fl: flowers, Fr: fruits; Rh: rhizome, A: ashes. Plants were sometimes used in a mixture: m_1: Herbal mixture made of C. febrifuga, S. latifolius, M. charantia, F. stipulosa, C. papaya / m_2: Herbal mixture made of P. schweinfurthii, M. charantia, S. longipedunculata / m_3: Herbal mixture made of C. frutescens and C. densiflorus / m_4: Herbal mixture made of S. anceps, S. occidentalis, Kavula mazumba, Mutamundele, Takange. Informant group: sp = specialist, nsp= patient; NI: information not indicated; conservation status according to IUCN red List: vulnerable[a] ,

Plant N°	Scientific name/local name*	Botanical family	Collection number	Part used	Modes of preparation	Usage (route, mode of administration)	Informant group[sp & nsp]	Use reports (UR)
1	*Nymphaea lotus L./*Longa dia simbi	Nymphaeaceae	2513	Wp	decoction	oral, drink	Sp	1
2	*Palisota schweinfurthii C.B.Clarke/* Mabunda bunda	Commelinaceae	894	L	decoction, maceration	oral, drink	Sp	3
				L	maceration	topical, facial wash		
				L	maceration[m2]	oral, drink & dermal, body massage		
3	*Entada abyssinica A.Rich./* Nsofi	Fabaceae	3468	R	decoction or maceration	anal, clyster & oral, drink	Sp	2
				R	direct use, grated fresh roots	topical, nasal drops		
4	*Brillantaisia owariensis P.beauv./* Malemba lemba	Acanthaceae	7925	L	maceration	oral, drink	Sp& nsp	2
5	*Sarcocephalus latifolius (Sm.)E.A.Bruce/* Nlolo	Rubiaceae	8231	R	decoction	oral, drink	Sp	1
6	*Fleroya stipulosa (DC.) Y.F. Deng[a]/* Nlongo	Rubiaceae	8227	T	decoction	oral, drink	Sp	1
7	*Crossopteryx febrifuga (Afzel.ex G.Don) Benth/* Mvala	Rubiaceae	8212	L	direct use, crushed fresh leaves	topical, ocular & nasal drops	Sp& nsp	6
				R	decoction[m1]	oral, drink		
				R	decoction or maceration,	oral, drink & anal, clyster		
8	*Trilepisium madagascariense DC./* Nsiekeni or nzeke nzeke	Moraceae	9795	R, Tb	decoction	oral, drink	Sp	1
9	*Steganotaenia araliacea Hochst. /* Kula mvumbi or Kula mpinga	Apiaceae	6116	R	infusion	oral, drink	Sp	1
10	*Daniellia alsteeniana P.A.Duvign/* Nlomba	Fabaceae	3512	T	decoction	oral, drink & facial washing	Sp	2
11	***Carica papaya L./* Papaya[Pt]	Caricaceae	5377	R	decoction[m1]	oral, drink	Sp	1

12	***Nicotiana tabacum L./* Fumu or Tobaco[Pt]	Solanaceae	7434	L	direct use, crushed fresh leaves	topical, ocular & nasal drops	Sp	1
13	*Vitex madiensis Oliv./* Nfilu	Lamiaceae	7186	L	direct use, crushed fresh leaves mixed with palm wine "maruvo"	topical, ocular drops	Sp	3
				L	decoction	oral, drink		
14	*Momordica charantia L./* Lumbuzu	Cucurbitaceae	8591	Ap	decoction[m1]	oral, drink	Sp	3
				L	maceration[m2]	oral, drink & dermal, body massage		
				L	decoction	oral, drink		
15	***Capsicum frutescens L./* Ndungu	Solanaceae	7404	S	direct intake	oral, chewing	Sp & nsp	2
				L	decoction[m3]	oral, drink		
				L	direct use, crushed fresh leaves	topical, cranial cataplasm		
16	*Securidaca longipedunculata Fresen./* Nsunda	Polygalaceae	4275	R	maceration[m2]	oral, drink & dermal, body massage	Sp	2
17	*Cymbopogon densiflorus (Steud.) Stapf/* Lusangu sangu	Poaceae	134	F, Fl	decoction[m3]	oral, drink	Sp	1
18	*Sansevieria hyacinthoides (L.) Druce/* Espada de S.Jorge	Asparagaceae	1110	L, R	direct use, grated fresh leaves & roots	dermal, body massage	Sp	1
19	*Aristolochia gigantea Mart./* Cipó mil	Aristolochiaceae	2174	R	infusion	oral, drink	Sp	1
20	*Cascabela thevetia (L.) Lippold/* Tevetive or Kizuza[Kim]	Apocynaceae	4105/A	Fr, L	infusion	oral, drink	Sp	1
21	*Smilax anceps Willd./* Mbuakaiona[Kim] or Lunzila nzila	Smilacaceae	1151	R	infusion[m4]	oral, drink	Sp	2
22	*Aframomum angustifolium (Sonn.) K.Schum./* Ginguenga	Zingiberaceae	1343	Rh, Fr	infusion	oral, drink	Sp	1
23	***Senna occidentalis (L.) Link/* Dinioka nioka or Kikunde[Kim]	Fabaceae	3536	R	infusion[m4]	oral, drink	Sp	1
24	*Monodora myristica (Gaertn.) Dunal/* Jimpeve	Annonaceae	2733	L	direct application, crushed fresh leaves	dermal, cranial cataplasm	nsp	1
25	*Ocimum gratissimum L./* Mansusu nsusu or Dinsusu nsusu or mansudi nsudi or matsudi tsudi	Lamiaceae	7366	L	decoction	oral, drink	nsp	2

				L, A	direct application	dermal, skin vaccine		
26	*Xylopia aethiopica (Dunal) A. Rich*/ Nkwua kwa	Annonaceae	2717	NI	NI	NI	nsp	1
27	*Chromolaena odorata (L.) R.M.King & h.Rob.*/ Mubulututu	Compositae	8816	L	direct consumption, crushed dried leaves	oral, chewing	nsp	1
28	***Bryophyllum pinnatum (Lam.) Oken.*/ Luyika yika	Crassulaceae	3166	L	direct use, crushed fresh leaves	dermal, body massage	Sp	1
29	*Gardenia ternifolia Schumach. & Thonn.*/ Lemba nzau	Rubiaceae	8285	Fr	decoction	oral, drink	Sp	1
30	*Azadirachta indica A.Juss.*/ Cura tudo [Pt]	Meliaceae	4176	L	direct use, crushed fresh leaves	dermal, body massage	Sp	1

3.4.3 Precaution of use, potential risk of use and recommendations

In order to identify potential risks of use associated with the herbal preparations described in this work, we inquired the users of FM (14 patients: 12 who chose FM as first treatment and 2 who turned to FM as second treatment) as well as the specialists' group, to find out if there was any precaution to be taken, when following the herbal treatment and if so, what these were.

All traditional practitioners except one listed at least one precaution of use. The three most cited were "no alcohol", "not for pregnant woman" and "with dietary restrictions" (see Table 3-4).

N°	Precaution of use	Frequency of citation (n=9)
1	No alcohol	8
2	Not for pregnant woman	6
3	With dietary restrictions	6
4	Restricted prescription for children	2
5	Not with modern treatment	2
6	No sexual activity	1

Table 3-4: Main precautions of use cited by healers when prescribing herbal remedies. An herbal remedy can be recommended with several precautions of use.

Less than half of the users (6 of 14) mentioned a precaution of use. Except for one patient that referred to dietary restrictions, all others precautions mentioned by the users could not be correlated to the ones stated by the specialists, e.g. to reduce physical effort during treatment period. Past experience and own knowledge also contribute to raise awareness of the risk of use of herbal preparations. So, for instance, one patient refused to take the plant-based prescription claiming that the recommended herbal preparation would give itching and affect the stomach.

3.5 Discussion

3.5.1 Use of folk medicine and health-seeking behavior in case of g-HAT

All interviewed patients underwent follow-up treatment in line with the Angolan National Sleeping Sickness Control Programs (NSSCPs). Our study revealed that 40 percent of the inquired patients had used herbal drugs before consulting a biomedical practitioner. This number appears high when we consider that the reference treatment is provided for free to HAT patients in the reference health centers. Comparable results were found in Senegal in a study led among tuberculosis patients (n=117), of whom 41 percent made use of herbal medicine before reference treatment [99]. This study shows that plant-based medicines play an important role in local processes of health care in Angola. With respect to this, Göhre et al. [87] found in their regional ethnobotanical assessment in the province of Uíge that out of 498 different use-reports for 122 plants, 72.1 percent referred to a medical use-reports. These findings provide ample evidence that folk medicine plays an important role in the current primary health care in Angola, even though the country has not yet approved a national policy on Traditional Medicine [100]. Thus the non-approved

status of folk medicine and its hidden space may push users not to disclose its use in front of biomedical staff, leading to an underestimation of its importance.
When we look at the key motivation of the FM group to consult a traditional practitioner as first line treatment, two reasons played a crucial role: "past experience with FM"and "family habits". Dos Santos (2012) in his master thesis conducted an explorative study in the pediatric hospital of Lubango (capital of Huila province in southern Angola) and analyzed the use of traditional medicine as the parents' response to their children's disease [101]. He found that 25 percent of the interviewed families were motivated to seek for a traditional treatment when based upon recommendation of the neighborhood or family habit. A study conducted in Kenya provided similar results, where reasons associated with the use of herbal medicine by patients in herbal clinics were analyzed [102]. Their results highlighted the importance of the impact of family, friends and neighbors on health care practices for herbal medicine. This underlines the meaningfulness of family tradition, community members and habits on health seeking behavior for herbal medicine.
Though our findings show a significant adherence to folk medicine among sleeping sickness patients (40 percent), the perceived effectiveness of the herbal treatment appeared to be low, with only 3 patients of 12 reporting satisfaction about improvement or alleviation of the symptoms. This could suggest that patients might turn to folk medicine more due to social habits or cultural aspects, like disclosing the cause of the disease, than as specific HAT-related health seeking practice. Nevertheless, being knowledgeable about medicinal plants appears to be an important reason associated with the use of folk medicine: More than half of our respondents were aware about the plant parts to be used, their preparation and partially about the name of the plants. This result is consistent with other studies [103], where the patients' knowledge and notion of medicinal plants influenced the use of herbal medicine.

Sleeping sickness is a chronifying disease that evolves in two phases. Stage 1 is the haemolymphatic stage whose leading signs are nonspecific as for example chronic and intermittent fever, headache, pruritus and lymphadenopathy. Stage 1 can even remain asymptomatic [1]. As the symptoms are unspecific, the patients often do not feel the need to go for a health check. Stage 2 is the meningoencephalitic stage that is characterized by the invasion of the nervous system by trypanosomes and marked by sleep disturbances and neuropsychiatric disorders. The specificity and severity of the symptoms in stage 2 push patients to seek treatment. Unfortunately, the second stage treatment presents frequent adverse reactions that can be severe or even life-threatening [2]. We have observed that HAT symptoms – evocated by patients and traditional practitioners – referred mainly to the second phase of this disease and were related to active health seeking. This finding is in line with a previous study conducted by Mpanya et al. among local population in Kasai-Oriental (Democratic Republic of Congo), where patients mainly seek for assistance in the late stage of the disease [104]. Moreover, it shows that the included traditional practitioners were aware of the signs of illness that correspond to the late stage. This suggests that the management of g-HAT in Angola only starts after the

outbreak of aggravating and apparent symptoms of the second phase, emphasizing the challenging situation traditional practitioners are confronted with.
The unspecific symptomatology in the early stage combined with neuropsychiatric disorders in the late stage of the disease account for the intricacy of a self-recognized illness. Indeed, the significant proportion of respondents who recourse to folk medicine also reflects that clinical signs of the disease may probably be related at first to a folk illness. Here, a folk illness designates an illness not equivalently associated to a biomedical disease, hence being considered locally as a "*traditional illness*". This local category of sickness is named "*doença tradicional*" ("traditional illness") in Portuguese and refers to a culture-specific understanding of an illness for which biomedical treatment does not help and whose cure is dependent on a "traditional treatment" provided exclusively by a traditional practitioner. For example, the traditional illness "*cabeça aberta"* that literally means "opened head" is a characterized by strong headache originating from midline along the sagittal suture and whose traditional treatment is made of a plant-based cataplasm applied on the cartilaginous cranial part. This result is comparable to other studies [102, 105], where respondents believe that certain characteristics of a perceived illness are traditionally recognized and categorized to be only cured by herbal medicine or traditional practitioners. Moreover, changes in personality and behavior that may occur in the second phase of sleeping sickness are mental signs that probably play an important role in relating the etiology to supernatural, magical or evil nature, which leads to recourse to a sorcerer ("*kimbandeiro")* or traditional practitioner. We conclude that a persistent non-specific symptomatology in the first stage as well as the prolonged neurological alterations in the second stage of the sickness account for the convolution of the patients' therapeutic itinerary.

The distance to the next reference health center was pointed out to be another limiting reason regarding biomedical treatment. In its g-HAT endemic provinces, Angola is equipped with 17 fixed health facilities (see Figure 1/red stars), distributed between 7 provinces (Luanda (1), Bengo (1), Zaire (5), Uíge (3), Kwanza Norte (5), Kwanza Sul (1), and Malanje (1)) for diagnosis (with the Card Agglutination Test for Trypanosomiasis [CATT tests]) and delivering reference treatment. In the case of the northern province Uíge, whose area covers 58'698 km^2 and whose population counts 1.5 million [12] there are only three health centers that can meet the needs of infected patients (at the time of the study). The distance for rural communities to one of these three reference centers lies between a travel-time of 1 to 3 hours [106], which causes considerable travel, accommodation and food expenses. Even though active finding strategies are run punctually in inland areas, the difficult accessibility to the reference treatment sites may have contributed to the use of FM in the management of sleeping sickness. On the other hand, traditional practitioners are located within the community area. By this, they are readily available and reachable local health providers. In this respect, it was estimated that the ratio of traditional practitioners to the population in Africa is 1:500, compared to 1:40'000 for medical doctors [20]. A new regional diagnostic project including the northern border zone of Angola (provinces of Zaire and Cabinda) is currently being tested

and analyzed. It is based on passive screening aims at detecting cases in early phase by the combination of three new diagnostic tests, namely the rapid diagnostic test (RDT), the fluorescence microscopy LED (FM-LED), and loop-mediated isothermal amplification of DNA [LAMP] [107]. These novel screening tests are implemented as routine tests in all health care units and any new patient should undergo this screening procedure. This new diagnostic strategy aims at detecting early phase cases and minimizing the distances by offering novel screening tests in all health care units. Reducing distance to treatment centers directly improves affordability of the treatment by reducing indirect costs like transport and food expenses.

A considerable amount of research has been conducted on health seeking behavior of patients suffering from chronic diseases like TB or HIV [108-116]. Few studies investigated health seeking behavior of patients with sleeping sickness [104, 117, 118]. In a study run in Uganda among sleeping sickness patients, Odiit et al. mentioned that only one patient of 119 (0,8%) "admitted that he had first seen a traditional healer"(p.343) before visiting a medical health facility. A second study examined the health seeking behavior of 66 HAT-patients in the Democratic Republic of Congo and showed that 23 (34%) had first consulted a "unqualified private practitioner "(p.871), without further specification on the type of local care provider [118]. Our exploratory study points out for the first time an important frequency of use (40%) of folk medicine before medical treatment of HAT.
To get a deeper understanding of the causes that exert an impact on the health seeking behavior for sleeping sickness in Angola, detailed investigations on the patients' therapeutic itineraries have to be carried out. Aspects to be included are among others constraints and prohibition associated to reference treatment [119] or drug toxicity and costs [120]. In the Democratic Republic of Congo, these were identified as significant factors which lead to a reduction of non- or low-adherence of local communities to medical prevention and treatment programs.

3.5.2 Ethnomedical use and antitrypanosomal activity of the studied plants

Of the 37 reported plants, 7 could not be identified because of lack of complete specimen[25]. This highlights the challenge of ethnopharmacological research. A botanical student of University of Agostinho Neto (Luanda) aided as a plant collector, and did several field walks in order to gather missing parts of specimen. Nevertheless, not all reported plants could be collected in a complete set, due for example to limited availability of respondents, time or financial constraints. Regarding conservation status, poor availability of data made it difficult to assess the potentially endangered species. Nevertheless, consultation of IUCN Red List (https://www.iucnredlist.org/) indicated *Fleroya stipulosa (DC.) Y.F. Deng* as being vulnerable and the book by [121] that covers this subject didn't point out any of the studied species as being threatened.

[25] The plant material will be used for future molecular identification

In view of the botanical species cited and the low number of known reported plants by the patients group (only 7 plants were mentioned), the distribution of knowledge of plants used to manage trypanosomiasis and its symptoms seems to be rather a specialized knowledge belonging to specialists group rather than to lay people. This aspect can be supported by the results obtained from the study of [87], where out of 82 individuals surveyed in Uíge province of Angola and 30 different use-reports for different disorders mentioned, none referred to sleeping sickness. This shows that the knowledge of specific herbal remedies is rather in hand of a small circle of specialists, what is not surprising due to complex signs and issues of this parasitic disease. As can be seen from Table 2, a large part of the plants were mentioned only by a single informant, and few plants were cited twice or threefold. It highlights the complexity of the disease and could indicate that there is no plant that obviously responds to this sickness. It can also be explained by the explorative character of the study and the small sample size of the informants groups. Another reason for the weak interrelationship between used plants and specialists could be the secrecy of local knowledge and family heritage. However, one plant, namely *Crossopteryx febrifuga* has gathered the most votes (6 Use-Report) and was mentioned by both groups of informants. The plant is known for its febrifuge, antitussive, antidiarrheal and analgesic proprieties [122]. Among the different herbal preparations this plant was mentioned in this work to be applied as a juice arising from the crushed leaves into the eyes and the nose in order to "clean the eyes and reestablish a blurred vision" and "fight the sleepiness". The decoction of the roots was administered as a tonic drink enabling fighting the great tiredness that accompanies the disease. Interestingly, 2 studies reported a similar route of administration in Congo (Brazzaville). First, [123] mentioned that the "*liquid is administrated into the nose for headache and into the eyes for the conjunctivitis caused by filariae*". Another information arising from this source is that the leaves are "*taken as tonic, and they are put into some prescriptions for mental trouble*". Moreover, it is said that the plant is also used against epilepsy, which is related to convulsion, a symptom which can appear in the second stage of the disease. In the second study, [124] led a survey on analgesic and psychotropic plants in Congo (Brazzaville) and their findings confirmed the ethnomedicinal use of *C. febrifuga* for these effects, depicting even the fact that the leaves of *C. febrifuga* were specifically used for their psychotropic properties, contrary to the roots which were administered as an analgesic remedy. At the time of Belgian Congo, the maceration of the bark of *C. febrifuga* was reported to be used against trypanosomiasis [125]. In Mozambique, the roots are taken as a febrifuge [123]. In Angola, a recent large-scale ethnobotanical study conducted in northern province of Uíge [90] reported *C. febrifuga* to the use-category "madness". Out of these various ethnomedicinal practices from Angola and neighbouring countries, *C. febrifuga* is confirmed to be a medicinal plant which is taken as a tonic, pain reliever, febrifuge, against mental disorder or epilepsy. These particular symptoms are encountered in the course of sleeping sickness, thus using a medicinal plant known to treat such ailment makes sense. In the light of the above mentioned aspects, the ethnomedicinal use of *C. febrifuga* finds support in traditional practices and *in vitro* studies confirmed the analgesic and antipyretic activity of the plant [126]. Glycosides flavonoids have been isolated from the

leaves of *C. febrifuga* [127]. A large study assessing flavonoids and their analogues for their antitrypanosomal activity [128] suggests that *C. febrifuga* glycoside flavonoids could have a similar activity.

Momordica charantia, *Palisota schweinfurthii* and *Vitex madiensis* were each mentioned by three different informants. *M. charantia*, also known as bitter lemon, is famous for its effectiveness against diabetes [129, 130]. Traditional preparations are commonly used for external applications in wounds healing and skin diseases and internally as a remedy to treat worms and menstrual problems [131, 132]. In South Africa a decoction of the leaves is recommended to act on the blood and sugar level [133], in Ivory Coast as an antimalarial as well as antidiabetic remedy [134] and in Angola as a febrifuge [87]. Among the various medicinal practices, the plant was never reported in the management of sleeping sickness. The same occurs for *Palisota schweinfurthii,* for which no reports against trypanosomiasis could be found. In folk medicine, *Vitex madiensis* has been reported to fight malaria in Gabon [135] and Mali [136] and schistosomiasis in Burkina Faso[137]. In Nigeria, Nwodo et al. mentioned its use against sleeping sickness without clearly describing the mode of preparation and usage [138]. A related species, *Vitex ferruginea* was described in a traditional preparation against sleeping sickness.[139] and the maceration of the leaves is used as a head bath in case of psychosomatic troubles in Congo (Kinshasa) [140]. In our work, *Vitex madiensis* was reported to be used directly, by applying ocular drops arising from the squeezed leaves mixed with local alcoholic beverage called “maruvo” and made of palm wine. Interestingly, *in vitro* investigations confirmed the presence of bioactive flavonoids in the alcoholic extract of the leaves, thus providing preliminary evidence of the antitrypanosomal potential of this traditional preparation. [138, 141]. From the foregoing, the recourse to *Crossopteryx febrifuga* and *Vitex madiensis* in the management of sleeping sickness and its symptoms could be correlated to a medicinal practice in use. On the contrary, the use of *Palisota schweinfurthii* and *Momordica charantia* couldn’t be corroborate by ethnomedicinal data.

Looking further at the cited plants in Table 3-2, seven species have seen their medicinal usage against sleeping sickness being supported by ethnobotanical surveys, namely *Azadirachta indica*, *Senna occidentalis*, *Securidaca longipedunculata*, *Entada abyssinica*, *Sarcocephalus latifolius*, *Brillantaisia owariensis* and *Nicotiana tabacum*. The leaves of the latter in association with *Boswellia dalzielli* and *Adenium obesum* were given to fight “nagana” (animal trypanosomiasis) in Nigeria’s Kaduna state [142] and alone in southern Ethiopia [143]. In our work, the juice of the fresh crushed leaves of *N. tabacum* was prescribed as nasal and eye drops by one of the inquired specialist, claiming it “awakes” the sufferer” and “alleviates headaches”. Similar herbal prescription was found in Congo (Brazzavile) where the leaves juice of *N. tabacum* is mixed to the one of *Solanum lycopersicum* to fight trypanosomiasis [144]. In addition, the leaves of *N. tabacum* are recommended for two HAT-related symptoms, namely strong headache (migraine) and epilepsy, in traditional practice in Equateur province of the Republic Democratic of Congo [140]. However, *N. tabacum* has never been investigated for its antitrypanosomal activity. *Sarcocephalus latifolius*, commonly called African peach, is a typical savannah tree, largely used throughout tropical regions for diverse medicinal purposes. Besides its well-known

usage against malaria and fever, in Angola the roots are found in local markets of Uíge province and sold as tonic [90]. Concerning trypanosomiasis, one old work revealed the use of this plant against the disease in Ivory Coast [145] and its roots bark was mentioned in Mali to treat this affection [146]. *Brillantaisia owariensis* is described in a polyherbal preparation made of 2 other plants as a remedy to fight trypanosomiasis in Equateur province of the neighbor country Congo (Kinshasa) [140]. In Angola, the plant is mentioned in the large-scale ethnobotanical survey led in province of Uíge [90], mainly to fight heart problem, blood pressure and it is several times cited against headache and also against epilepsy and madness. These findings corroborate ours, where *B. owariensis* was prescribed to treat the suffering of headache, madness and convulsions, all symptoms induced by this disease. *Entada abyssinica*'s medicinal use in local herbal preparation has been reported twice from the southern region of Uganda. In both studies, the roots are prescribed, either after infusion or decoction, as an oral administration to fight trypanosomiasis [147, 148]. In Angola, usage of *E. abyssinica* in the management of sleeping sickness was not strictly confirmed by another study. However, the roots were cited to be used against two HAT-related symptoms, that are epilepsy and headache [90]. *Securidaca longipedunculata, Senna occidentalis* and *Azadirachta indica*, three important multipurpose plants in African traditional medicine, have been mentioned in herbal remedies against human or animal sleeping sickness in Senegal [149], Congo Brazzaville [150] and in Nigeria [151] respectively. Moreover, the roots of *S. longipedunculata* are generally used against headache, fever and convulsion, all HAT- related symptoms [152]. Interestingly *B. owariensis* , *Vitex madiensis and Securidaca longipedunculata* seem to be used in the cure of mental suffering arising from supernatural or evil cause [153, 154]. In this context, their use echoes mental or behavioral disorders occurring in the second phase of the disease and underlines its association with supernatural or magical forces. Of 30 plants mentioned in our study, our ethnobotanical literature search provided support for the ethnomedical claim for 8 species with a reliable concordance of the plant part used. Other plants like *Ocimum gratissimum* or *Nymphaea lotus*, haven't been cited in ethnobotanical studies against trypanosomiasis per se, however their usage covers several HAT-related symptoms like strong headache, fever, convulsion or mental disorders. Therefore, about one third of the reported plants in this work could be correlated with the ethnomedicinal practice against sleeping sickness and its symptoms.

The antitrypanosomal potential of African medicinal plants and natural compounds has been reviewed in the last decade by [47], [48], [155] and [156]. Starting from there, a literature review of the studied plants revealed that out of the 30 identified species, 17 (56%) had their antitrypanosomal activity supported by in vitro and/or in vivo studies (see Table 3-5) namely *Nymphaea lotus, Entada abyssinica, Sarcocephalus latifolius, Crossopteryx febrifuga, Carica papaya, Vitex madiensis, Momordica charantia, Securidaca longipedunculata, Cymbopogon densiflorus, Smilax anceps, Senna occidentalis, Monodora myristica, Ocimum gratissimum, Xylopia aethiopica, Chromolaena odorata, Bryophyllum pinnatum, Azadirachta indica.* Of this panel of 17 plants, *C. febrifuga* is the only one that was mentioned by specialists and patients, whereas *O. gratissimum*, *X. aethiopica*, *C.*

odorata and *M. myristica* are the 4 species reported by the patients only. The 12 remaining plants were mentioned specifically by the involved specialists. The pharmacological results of the more promising plants of this work are discussed in the next paragraph according to the type of extract and plant part used. We have to consider that the traditional remedies of the studied plants were mainly prepared as a decoction, infusion or maceration, all water-dependent preparations. Therefore, a special focus will be given on aqueous extracts as the most reproducible traditional type of extract, while assessing the antitrypanosomal activity in the selected references.

The plant candidate that matches best between preclinical results and the traditional preparation mentioned in this work, is *Ocimum gratissimum* a medicinal plant mentioned only by patients and reported for its antitrypanosomal activity in 10 studies (see Table 5) This tropical aromatic plant, commonly named African basil, is widely spread and easily accessible in Angola for the local population who uses it either as condiment or remedy against diarrhea, stomach disorders, urinary infections, headaches, coughs and bronchitis [93]. The essential oil of *O. gratissimum* has demonstrated antibacterial and antifungal activity and its major compound eugenol showed promising antiprotozoal activity when assayed against the amastigote and promastigote forms of *Leishmania amazonensis* [157]. In our work, the traditional preparation of *O. gratissimum* was made of fresh leaves or boiled in decoction. Two *in vitro* studies [158, 159] and two *in vivo* assays [158, 160] tested the aqueous extract of the leaves of *O. gratissimum against Trypanosoma brucei spp.*. The *in vitro* results were variable with one study demonstrated an inhibitory activity (IC_{50} = 7.58 µg/ml against *T.b.brucei*) as to the *in vivo* results, which at best reported prolonged time of survival of the infected animal. The most interesting *in vitro* outcome was obtained with the ethanol extract of the leaves assayed against *T.b.brucei* which exhibited an IC_{50} value of 1.66 µg/ml without being cytotoxic (SI: 12.89)[161]. From the foregoing, the use of the leaves of *O. gratissimum* prepared traditionally as a decoction to fight sleeping sickness is supported by these pre-clinical data.

A second plant intensively investigated and which counts in all 8 studies assessing its antitrypanosomal potential is *Azadirachta indica.* The neem or Margosa tree, a tropical evergreen plant, is known since ages in the Indian systems of medicines and considered as the "wonder tree" being an invaluable source for a variety of medicinal properties [162-164]. In folk medicine the neem drug is used for various ailments such as inflammatory and febrile disease, cutaneous affections, measles, smallpox, earache, stomachache and burning sensations. The antimalarial use of the leaves and stem bark has been reviewed [165, 166], some studies reporting antimalarial effect [167-169] and others being less clear [170]. Even though folk medicine doesn't mention *Azadirachta indica* against human sleeping sickness, nevertheless pharmacological investigations demonstrated that the alcoholic extracts of the leaves and stem bark of this plant obtained most promising *in vivo* outcomes with a complete parasitemia clearance in the infected animal after more than 30 days post infection [171, 172]. In our work, the use of leaves of *A. indica* was made as a prevention remedy. Thus, a traditional practitioner reported its repellent action by rubbing fresh leaves on the flies-exposed parts of the body. Different studies confirm its use as a natural fumigant to repel mosquitoes [173, 174] or as an herbal preparation made

of leaves applied directly on the skin to treat various skin disorders like scabies, urticaria, eczema or skin infections [162]. Neem oil made out of seed kernels extract was tested on rabbits' skin against *Aedes aegypti* mosquitoes and proved some repellent action without providing complete protection [175]. A field study in Ethiopia on human volunteers confirmed the repellent efficacy of neem oil during 3 hours against *Anopheles arabiensis* [176]. However, a direct application on the skin of the juice of the crushed leaves was never reported to date as a natural repellent. Azadirachtin, a bioactive triterpenoid of *A. indica* found in the seeds, leaves and bark has been positively correlated with the insecticidal property of neem oil. The presence of azadirachtin in the leaves could be responsible for the repellent action of the reported preparation which is directly applied on the skin. From the foregoing, the use of the leaves as a repellent has a proven rational and the very encouraging results obtained with the methanol extract of the leaves together with the stem bark extract should be considered for further investigation in the search for a novel drug candidate against trypanosomiasis.

Another plant that gathered most promising in vivo results is *Nymphaea lotus*. This plant, also called water lily, is an aquatic plant with white flowers widely spread in tropical Africa. In our work, the plant was reported to have served as an herbal remedy to fight sleeping sickness during epidemic periods in Uíge province. Traditional herbal preparations made of *N. lotus* are mentioned in case of cancer [177], or stomach ulcers or as narcotic and sedative [178]. It is prescribed in combination with other plants species to treat "tazo" (malaria) in the eastern region of Madagascar [179]. To the best of our knowledge, the recourse to water lilly in the preparation of a local remedy against sleeping sickness could not be confirmed. Nevertheless, [180] provided very promising antitrypanosomal *in vivo* results with a 70% methanol extract of *N. lotus* reducing parasitemia in infected mice with *T.b.brucei* at a dose of 100mg/kg/day. The phytochemical profile of the methanol extract revealed the presence of saponins, tannins, cardiac glycosides, and phlobatannins without identifying a bioactive constituent. Due to its *in vivo* antitrypanosomal potency, additional pharmacological studies should explore the antiprotozoal potential of *N. lotus*.

Three more species were assessed for their antitrypanosomal activity by more than five preclinical studies for each, namely *Entada abyssinica*, *Sarcocephalus latifolius* and *Securidaca longipeduncunlata.* Among the different studies, few tested specifically the aqueous extract of the plant's part used in the traditional preparation and only the aqueous extract of the roots of *S. longipedunculata* exhibited two positive *in vivo* results against *T.brucei brucei* with a transitory reduction in parasitemia [181, 182]. Moreover, Abubakar et al. showed that the extract improved hematological parameters in the infected rats. However, the aqueous extract of the roots *S. longipeduncunlata* when assayed *in vitro* against the same parasite wasn't active [146] what suggests that inactive *in vitro* precursor constituents may be metabolized in active substances *in vivo*. Root decoction of *Entada abyssinica* was correlated with a moderate outcome among the pharmacological data (MIC ≤ 56 µg/ml), nevertheless the dichloromethane extract of the rootbark demonstrated very encouraging *in vitro* results with an IC_{50} ≤ 0,5 µg/ml and isolation of a bioactive compound named diastereoisomer of kolavenol (IC_{50} = 2,5 µg/ml)

[183, 184]. Concerning *S. latifolius*, the variable results of the antitrypanosomal activity of the root extracts request for further investigation to clarify the inhibitory potential of this plant against *Trypanosoma brucei ssp*.

As we have seen from its ethnomedicinal uses, *Crossopteryx febrifuga* has various medicinal proprieties, which resulted in a wide range of pharmacological studies. However, few have investigated its trypanocid potential. Considering the leaves' part, only one study assessed the antitrypanosomal activity of the methanolic extract against *T.b.brucei* which resulted in a weak inhibitory activity (IC_{50} = 39±3.3 µg/ml)[185]. The roots were not analyzed for now. As the curative proprieties of the leaves and roots are correlated with the relief of several HAT-symptoms, it would be worth investigating this plant for its antitrypanosomal potency.

Other plants like *Vitex madiensis*, *Momordica charantia*, *Smilax anceps* and *Chromolaena odorata* have obtained positive *in vitro* results for their alcoholic extract of the indicated plant's part against *Trypanosoma brucei ssp*. Artemetin, a flavonoid, was even isolated from the methanol extract of the leaves of *V. madiensis* and exhibited an IC_{50} value of 4.7 µg/ml against *T.b.rohdesiense*. Nevertheless, the aqueous extracts of these 3 species were not assessed so far. Considering the polarity range of the methanol extracts, we cannot exclude that an aqueous extract of the same plant part would show an inhibitory activity. Therefore, the antitrypanosomal potential of the reported traditional preparation for each of these 3 species should be assessed.

Of the 17 referenced plants, 3 species, namely *Monodora myristica, Cymbopogon densiflorus* and *Carica papaya*, haven't seen their traditional usage confirmed either by ethnobotanical surveys or by comparison with preclinical data.

When assessing plant candidates issued from traditional preparation, it should be kept in mind that the herbal treatment prescribed by the traditional practitioners was made of one to three recipes which comprises one or more different plants. Thus, the potential synergistic effect due to the plant combination is at preclinical level almost never taken into account. Furthermore, results discrepancy for a same extract tested against the same parasite model can be explained by the differences in study protocol, in phytochemical profile of the plant material. Consequently, the outcome of such a comparative analysis among various studies, which are realized in different countries, must be considered with precaution.

Nevertheless, if we consider the specific plant part used and the type of extract tested few of the reported medicinal plants match to the described antitrypanosomal activity. Among the 17 referenced plants, only 3 species, namely *Entada abyssinica*, *Securidaca longipeduncunlata* and *Ocimum gratissium* showed *in vitro* or/and *in vivo* evidence to corroborate the antitrypanosomal potential of the reported traditional preparation. In term of prevention, the traditional use of *Azadirachta indica* as a repellent could also be associated with referenced literature. *Ocimum gratissimum* and *Nymphaea lotus* displayed very promising *in vivo* results for non-aqueous extracts type with a complete parasitemia clearance. *Vitex madiensis*, *Momordica charantia*, *Smilax anceps*, *Chromolaena odorata* and *Crossopteryx febrifuga* lack assessment of the aqueous extract of the recommended plant component and should be therefore complemented. *Palisota schweinfurthii* which was

cited 3 times and for which no HAT-related information is available should be investigated further

Table 3-5: Main representative references of the reported plants and their antitrypanosomal activity.

Column title abbreviations: IC_{50}: half maximal inhibitory concentration; MIC: minimum inhibitory concentration; cpd: compound; Plant parts' abbreviations: R: root; B: bark; SB: stem bark; RT: root bark; L: leaves; WP: whole plant; Aep: aerial part; S: stem; RH: rhizome. Solvents' abbreviations: Aq: aqueous; MeOH: methanol; PE: petroleum ether; DCM: dichloromethane; EtOH: ethanol; Hex: hexane; EtoAC: ethylacetate; $CHCL_3$: chloroform; Aq.(EO): aqueous fraction of essential oil. Country abbreviation: RDC: Republic Democratic of Congo. Extracts' fractions abbreviations: MeOH_F: methanolic fraction; PE_F: petroleum ether fraction; DCM_F: dichloromethane fraction; MeOH_NH4OH_F: methanol ammonium hydroxide fraction; MeOH VLC_DCM_F: methanol vacuum liquid chromatography dichloromethane fraction E; MeOH_VLC_EtOAc: methanol vacuum liquid chromatography ethylacetate extract; EtoAc_F: ethylacetate fraction; $MeOH\_FIII^{L}$: methanol extract fraction III: MeOH_F3: methanol extract fraction 3. Parasites models' abbreviatiions: T.b.r: Trypanosoma brucei rhodesiense; T.b.b: Trypanosoma brucei brucei; T.cruzi: Trypanosoma cruzi; T.cg: Trypanosoma congolense; T. evansi: Trypanosoma evansi; H.s: Herpetomonas samuelpessoai. Compound number: 1: diasteroisomer of kolavenol; 2: monomethyl ester-15-kolavic acid; 3: naucleidinal alkaloid; 4: carpaine; 5: artemetin: 6: 1H-indol-5-yl)methanol; 7:-4-(1H-indol-5-yl)but-3-en-2-one; 8:15-oxo-ent-kaur-16-en-19-oic acid; 9-11: tetranotriterpenoid. Categorisation of in vivo activity: 1: no activity; 2: transitory decrease in parasitemia; 3: complete parasite clearance. NA means that the results were not expressed as IC_{50} values or MIC, thus are not available. The selectivity index (SI) was reported in the table, when it was given in the referenced literature.

STUDIED PLANTS	REPORTED IN VITRO ANTITRYPANOSOMAL ACTIVITY			REPORTED IN VIVO ANTITRYPANOSOMAL ACTIVITY			REPORTED IDENTIFIED ACTIVE COMPOUND				REFERENCES
Scientific name[part used]	Type of extract [plant part]/ country	parasite	IC_{50} or MIC [µg/ml]	Type of extract[plant part]/ country	animal & parasite	activity	Type of extract [plant part]/ country	parasite	cpd	IC_{50} or MIC [µg/ml]	
Nymphaea lotus L., ^{Wp}				MeOH70%^{Wp} / Nigeria	T.b.b & mice	3					[180]
Entada abyssinica A.Rich^{R}	Aq.^{R} / Uganda	T.b.r	≤ 56^{a}								[148]
	MeOH^{R} F/Uganda	T.b.r	3.3 ±1.3								[148]
	PE^{R} F/Uganda	T.b.r	≤ 0.4 ±0.2								[148]
	DCM^{R}_F/ Uganda	T.b.r	≤ 0.5 ±0.3				DCM^{R}/ Uganda	T.b.r	1	2.5 ±0.2	[148, 184]
	EtOH^{B} / Ivory coast	T.b.r	9								[186]
							DCM^{SB}/ Cameroon	T.b.b	2	1.7 µM	[187]
	MeOH^{SB}/ Tanzania	T.b.b	46.39 (SI:3.24)								[188]
	DCM^{SB} / Tanzania	T.b.b	56.21 (SI:1.85)								[188]
				EtOH^{RT}/ Tanzania	T.b.b & mice	2					[189]
Sarcocephalus latifolius (Sm.)E.A.Bruce^{R}				EtOH^{RT}	T.b.b & mice	2					[190]
				Aq.^{R}	T.b.b & mice	1					[191]
	MeOH^{R} / Nigeria	T.b.b	>500								[192]
	MeOH^{RB}, DCM^{RB}/ Mali	T.b.b	≤ 10^{a}								[146]
	Aq.^{SB}, MeOH^{SB} / Nigeria	T.c	NA								[193]

	Hex, EtoAc, MeOH^{B} / Nigeria	T.b.b	NA				EtoAc^{B} / Nigeria	T.b.b	3	12.5^{a}	[194]
				Aq.^{L}/ Nigeria	T.b.b & rats	1					[195]
				Aq.^{SB}/ Nigeria	T.b.b & rats	2					
				MeOH^{SB}/ Nigeria	T.b.c & rats	2					[196]
Crossopteryx febrifuga (Afzel.ex G.Don) Benth ^{L,R}				Aq.^{SB}/ NA	T.b.c & rats	2					[197]
	MeOH80%^{L} ,RDC	T.b.b	39±3.3 (SI:>1.6)								[185]
		T.cruzi	> 64								
****Carica papaya*** ^{L,R}				CHCl3^{S}/Mexico	T.cruzi & mice	2					[198]
							MeOH-NH4OH^{L}/ Indonesia	T.b.r	4	12.7µM	[199]
								T.cruzi		16.3µM	
Vitex madiensis Oliv. ^{L}	MeOH^{L}/ Nigeria	T.b.r	14.2								[200]
							MeOH_VLC_DCM_F^{L}/ Nigeria	T.b.r	5	4.7 (SI: 9.8)	[138]
Momordica charantia L. ^{AeP}	MeOH80%^{WP}/RDC	T.b.b	7±1.3 (SI:0.4)								[185]
	EtOH95%^{L}/ Brasil	T.cruzi	46.06								[201]
	Fruit (Bitter lemon)	T.brucei	NA								[202]
Securidaca longipedunculata Fresen. ^{R}	MeOH^{RB} and Aq.^{RB} / Tanzania	T.b.r	56^{MIC}								[183]
	MeOH^{R}/ Nigeria	T.b.b, T.cg	NA								[203]
	Aq.^{R}, MeOH^{R} , DCM^{R}/ Mali	T.b.b	not active								[146]
	DCM^{R}/Mali and Brukina faso	T.b.b	50^{a} (SI >10)	DCM^{R}/Mali and Brukina faso	T.b.b & mice	2					[204]
	DCM^{NI}/ Tanzania	T.b.b	11.20 (SI:12.03)								[188]
				Aq.^{R} /Nigeria	T.b & rats	2					[182]
				MeOH_VLC_EtOAc^{R}/Nigeria	T.b.b & mice/rats	2					[205]
				Aq.^{R} /Nigeria	T.b.b & rats	2					[181]

	MeOH[R]/ Nigeria	T.b.b	5[a]	MeOH[R]/ Nigeria	T.b.b & rats	2					[206]
	MeOH[SB], EtoAc[SB],Aq.[SB], /Nigeria	T.b.b	NA	MeOH[SB], EtoAc[SB],Aq[SB], /Nigeria	T.b.b & rats	1					[207]
				MeOH[SB], EtoAc[SB],Aq[SB], /Nigeria	T.b.b & rats	2					[208]
Cymbopogon densiflorus (Steud.)Stapf [Fr, Fl]	MeOH80%[WP]/ Congo	T.b.b	33 ±1.3								[185]
Smilax anceps Willd. [R]	MeOH90%[RH] / Ivory coast	T.b.r	> 25								[186]
****Senna occidentalis (L.) Link*** [R]	EtOH95%[L]/ Nigeria	T.b.b	NA	EtOH95%[L]/ Nigeria	T.b.b & rats	2					[209]
				EtOH95%[L]/ Nigeria	T.b.b & mice	2					[210]
Monodora myristica (Gaertn.) Dunal [L]	DCM[S]/ Ivory coast	T.b.b	NA								[211]
	EtoAc F[S]/ Nigeria	T.b.b	3.125[a]				EtoAc_F[S]/ Nigeria	T.b.b	6	25[a]	[194]
									7	12.5[a]	
Ocimum gratissimum L. [L]	Aq(EO)[L] /Brazil	H. s.	91								[212]
	MeOH80%[WP] /RDC	T.b.b	32 ±3.4 (SI:0.4)								[185]
		T. cruzi	26 ±2.7 (SI:0.5)								
	Aq.[L] / Nigeria	T.b.b	NA	Aq.[L] / Nigeria	T.b.b & rats	2					[158]
				Aq.[L] /Nigeria	T.b.b & rats	1					[160]
	EtoAc[L]/ Nigeria	T.b.r	2.08 ±0.01 (SI:29)								[213]
	Aq.[L] /RDC	T.b.b	7.58 (SI>8.4)								[159]
		T. cruzi	30.52 (SI>2.1)								
	Aq(EO)[Ni]/ Brazil	T. cruzi	11.5 (SI>15.7)								[214]
				70%EtOH[S]/ Nigeria	T.b.b & mice	2					[215]
	EtOH[L] / Benin	T.b.b	1.66 ±0.48 (SI:12.89)								[161]
	EtOH[S] /Benin	T.b.b	1.29 ±0.42 (SI:10.94)								

Xylopia aethiopica (Dunal)							EtoAc_F[FR]/ Cameroon	T.b.b	8	27.04± 0.03 µM	[216]
Chromolaena odorata (L.) R.M.King & h.Rob.[L]	MeOH[L] /Malaysia	T.b.b	>12 (SI:nd)								[217]
****Bryophyllum pinnatum (Lam.) Oken.[L]***	Aq.[L] / Nigeria	T. evansi	NA								[218]
		T. cg	NA								
	DCM[L], MeOH[L]	T.b.r, T.cruzi	NA								[219]
Azadirachta indica A.Juss.[L]				MeOH_FIII[L]/ Nigeria	T.b.b & rats	3					[171]
	$CHCl_3$[L]/ Venezuela	T. cruzi	NA								[220]
				DCM[B] / Kenya	T.b.r & mice	2					[221]
				Aq.,DCM, MeOH[SB]/ Kenya	T.b.r & mice	1					[222]
	$CHCl_3$_F[L]/ Kenya	T.b.r	4.4±0.0[a]				$CHCl_3$_F[L]/ Kenya	T.b.r	9	6.9	[223]
									10	15.6	
									11	7.8	
	EtOH[SB]/ Nigeria	T.b.b	NA	EtOH[SB]/ Nigeria	T.b.b & rats	3					[172]
	MeOH[S] /Nigeria	T. evansi	NA	MeOH_F3[S] /Nigeria	T.evansi & rats	2					[224]
	Aq.(EO)[L]/Cameroon	T.b.b	15.21 ±0.97 (SI>6.57)								[225]

[a] reported MIC values

3.5.3 Potential undesired or toxic side effects

Herbal medicines lack of standardization, and very few prescribed remedies have been rigorously tested for their toxicity, especially for their long-term effect. For this reason, it is important to address the potential risk of use of the studied plants, and report it to the concerned population. Several plants mentioned in this work raise important questions about potential toxicities.

Among these, tobacco (*Nicotiana tabacum*) and *Thevetia peruviana* could easily lead to acute toxicity accidents in case of overdoses. Tobacco plant is rich in nicotine, a powerful neurotoxic water-soluble alkaloid that is present in the leaves. *Thevetia peruviana* contains cardiotoxic glycosides in all parts of the plant, particularly in the sap and seeds. This widespread ornamental plant has been linked with numerous fatal intoxications in the tropics [226]. As the use of its fruits was mentioned by one of our specialist, awareness needs to be raised about the use of its herbal preparation. Even more concerning is the use of *Aristolochia gigantea*, since the whole genus Aristolochia has been identified as nephrotoxic and carcinogenic due to the presence of aristolochic acids and/or aristolactams in all parts of these plants [227]. Adverse effects can occur several weeks or months after intake, and such delayed and cumulative effects remain generally unsuspected by the users. *Securidaca longipedunculata* is another plant that should be suspected of potential danger [152, 228]. The fact that an interviewed traditional practitioner recommended an aqueous extract of the roots of this medicinal plant without even mentioning precaution of use for pregnant women points out the necessity to analyze the risk associated with the use of herbal preparations and to discuss it with the target population. Based on ethnobotanical reports, even if strong toxicological evidence is lacking, attention should be paid to herbal remedies containing *Sansevieria sp*. and *Smilax sp*. because both contain haemolytic saponins and are components of hunting poisons [229]. Similarly, *Capsicum frutescens,* although widely consumed as a spice, is not devoid of danger since some wild ecotypes are so rich in capsaicin and other pungent compounds that severe burns can occur [229]. The above mentioned plants highlight the need for a true pharmacovigilance in the field of traditional remedies, as emphasized by the World Health Organization [230]. We will organize a “feed-back session” with the concerned population (users and providers of FM) in order to share these concerns and look for alternatives.

3.6 Conclusion

The explorative ethnomedicinal study part shows evidence that three main factors – accessibility, cultural acceptability and affordability – account for the recourse to folk medicine in the management of g-HAT in the studied areas. The frequency of use of folk medicine for g-HAT is significant, 40% of the inquired patients resorted to folk medicine before receiving reference treatment. Recognizing local perspectives and practices of HAT management in Angola will be essential for a comprehensive understanding of dynamics in a local healthcare system.

In an ethnobotanical outlook, and though Angola faced several epidemic g-HAT periods, none of the traditional practitioners claimed to be a specialist in relation to sleeping sickness. More than half of the 30 identified botanical species have been previously reported for their antitrypanosomal activity, which supports the ethnopharmacological approach. The use of

herbal remedies seems not to be concomitant with the medical treatment, which reduces the risk of drug interaction between herbal drug and reference treatment.
This study shows supportive evidence for the ethnomedicinal use of some plant species as herbal treatment in the management of sleeping sickness. At the same time, some of the used plants raise serious concerns about toxicity. This work is a contribution to a more evidence based use of herbal remedies and a first step towards the validation of herbal preparations used in the management of trypanosomiasis in Angola and their potential usefulness.

ACKNOWLEDGEMENTS
We are very grateful to all the patients and traditional practitioners who invited us to enter their privacy, trusted us and shared with us a precious piece of their life experience and knowledge.
This research would not have been possible without the support of the former Direction of the National Center of Scientific Investigation (CNIC) and assistance of its scientific collaborators. Special thanks go to its former scientific Director, Dr. João Pedro Guilherme, whose advices, experience and determination facilitated the implementation of this field work. We would also like to thank the former Director of the ICCT, Prof. Dr. Josenando Théophile for giving the assurance of the need of this study, Dr. Amadeu and Dr. Paulo Makana for their support and Sr. Mafuta Junior and Sr. Felix Makiadi Donzoan for their constant disposability.
Many thanks are also due to the Coordinator of the CATEMATA, Sr. Kitoko Mayavângwa called *Papa Kitoko*, for helping me building trust with the traditional practitioners. I give thanks to the former Director of the FOMETRA in Uíge, Sr. António Kiakubua for his role as "gate opener" and his long-lasting support. A particular thank goes to PD Dr. Christian Schindler of the Swiss TPH who provided statistical guidance, to Ndombe Wandombe Filipe of ICCT who draw the maps, to Aristides of the CNIC who drove us safely into remote areas, to Dr. Amândio Luis Gomes from the UAN who helped us in the botanical identification of the plants' species and to Sr Mambote from Uíge for his most valuable translation competence.
We would also like to acknowledge all persons, administrative personnel, students who shared a piece of this experience and contribute to its fulfillment.
A very personal thank goes to my dearest soul and heart colleague Andreas Lardos for his most experienced advises and his constant encouraging words.
We would like to thank the anonymous reviewers for their very valuable comments.
Special thanks go to former Minister of Sciences and Technology, His Excellency Prof. Dr. Maria Cândida Texeira who made the implementation of this study work in Angola possible.

DECLARATIONS OF INTEREST
None. This research was partially granted by the Ministry of Science and Technology of Angola as part of a collaborative PhD research work with the Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, in Basel (Switzerland).

4 ANTIPROTOZOAL ACTIVITY OF PLANTS USED IN THE MANAGEMENT OF SLEEPING SICKNESS IN ANGOLA AND BIOACTIVITY-GUIDED FRACTIONATION OF *BRASENIA SCHREBERI* J.F.GMEL AND *NYMPHAEA LOTUS* L. ACTIVE AGAINST *T. B. RHODESIENSE*

Nina Vahekeni[1,2], Théo Brillatz[3], Marjan Rahmaty[3], , Monical Cal[1,2], Sonja Keller- Romina Rocchetti [1,2], Marcel Kaiser [1,2], Sibylle Sax [1,2], Kevin Mattli [4], Evelyn Wolfram [4], Laurence Marcourt [3], Emerson F. Queiroz [3], Jean-Luc Wolfender [3] and Pascal Mäser [1,2]

[1] Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, 4123 Allschwil, Switzerland

[2] Faculty of Science, University of Basel, 4002 Basel, Switzerland

[3] School Institute of Pharmaceutical Sciences, Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU - Rue Michel Servet 1, CH-1211, Geneva 4, Switzerland

[4] Zürich University of Applied Sciences (ZAHW), Phytopharmacy & Natural Products, Institute of Chemistry and Biotechnology, Wädenswil, Switzerland

Molecules 2024, 29, 1611

I collected and imported the draw plant material from Angola. I produced half of the plant extracts whereas Marjan Rahmati produced the other half. The antitrypanosomal activity testing on *Trypanosoma brucei rhodesiense* were carried out by myself. In collaboration with Théo Brillatz, I fractionated the active extracts and isolated the active constituents.

4.1 Abstract

Folk medicine is widely used in Angola, even for human African trypanosomiasis (sleeping sickness) in spite of the fact that the reference treatment is available for free. Aiming to validate herbal remedies in use, we have selected nine medicinal plants and assessed their antitrypanosomal activity. 122 extracts were prepared using different plant parts and solvents. 15 extracts from seven different plants exhibited in vitro activity (>70% at 20 µg/mL) against *Trypanosoma brucei rhodesiense* bloodstream forms. The dichloromethane extract of *Nymphaea lotus* (leaves and leaflets) and the ethanolic extract of *Brasenia schreberi* (leaves) had IC50 values ≤10 µg/mL. These two aquatic plants are of particular interest. They are being co-applied in the form of a decoction of leaves because they are considered by local healers as male and female of the same species, the ethnotaxon "longa dia simbi". Bioassay-guided fractionation led to the identification of eight active molecules: gallic acid (IC50 0.5 µg/mL), methyl gallate (IC50 1.1 µg/mL), 2,3,4,6-tetragalloyl-glucopyranoside, ethyl gallate (IC50 0.5µg/mL), 1,2,3,4,6-pentagalloyl-β-glucopyranoside (IC50 20µg/mL), gossypet-in-7-O-β-glucopyranoside (IC50 5.5µg/mL), and hypolaetin-7-O-glucoside (IC50 5.7µg/mL) in *B. schreberi*, and 5-[(8Z,11Z,14Z)-heptadeca-8,11,14-trienyl] resorcinol (IC50 5.3µg/mL) not described to date in *N. lotus*. Five of these active constituents were detected in the traditional preparation. This work provides the first evidence for the ethnomedicinal use of these plants in the management of sleeping sickness in Angola.

Keywords: ethnopharmacology; African medicinal plant; antiprotozoal; trypanosomiasis; Brasenia schreberi; Nymphaea lotus; Angola

4.2 Introduction

The extensive use of folk medicine in Africa, composed mainly of medicinal plants, is linked to cultural as well as economic reasons. This is why the World Health Organisation (WHO) encourages African member states to promote and integrate traditional medical practices in their health systems [26]. In Angola, 72%[26] of the population uses herbal medicines to treat various medical affections, including parasitic infections such as human African trypanosomiasis (HAT), also called sleeping sickness.

HAT is a vector-borne Neglected Tropical Diseases (NTD) that is transmitted by the bite of infected tsetse flies (*Glossina* spp.). HAT is caused by two subspecies of the protozoan parasite *Trypanosoma brucei*: *T. b. gambiense* in west and central Africa including Angola is responsible for the chronic form, whereas *T. b. rhodesiense* prevalent in eastern Africa causes the acute form [1]. Both forms are fatal if untreated. The majority of HAT-cases are of the gambiense form (g-HAT) and 57 million people are at risk of contracting g-HAT [231]. In Angola, g-HAT is endemic in the northwestern part. It is prevalent in seven of eighteen provinces [14]. It affects mainly remote rural communities, where the health infrastructure is basic and accessibility complicated [14].

Until recently, the chemotherapy of HAT relied on only five drugs, according to disease stage and parasite subspecies. This was unsatisfactory because the clinically available drugs had limitations such as toxicity, resistance, high cost, and parenteral administration [232]. The recent approval of fexinidazole as a new oral drug for both stages of g-HAT great-ly facilitates the treatment and will increase the coverage [233, 234]. The current reference treatment is available for free in Angola. Nevertheless, a previous ethnobotanical study reporting the use of local herbal remedies against sleeping sickness pointed out that 40% of the infected patients had recoursed first to herbal remedies before receiving the medical reference treatment [235]. Therefore, the investigation of herbal remedies is of high practical relevance. There have been several reports on the antitrypanosomal activity of traditionally-used African medicinal plants [47, 48, 77-83, 236, 237]; this is the first such study from Angola.

The laboratory results demonstrate that the medicinal plants in use to treat HAT possess antitrypanosomal activity. Bioassay-guided fractionation led to the identification of eight active molecules. Furthermore, the study provides evidence for the antitrypanosomal potential of a local preparation made of *B. schreberi* and *N. lotus* in the management of sleeping sickness in Angola.

4.3 Results and discussion

4.3.1 Selection of the candidate plants

In a previous ethnobotanical study, 30 species of medicinal plants had been identified in the management of sleeping sickness in Angola [235]. Pursuing the aim to further investigate the studied plants as potential candidates for this disease, we selected 9 species for pharmaco-

[26] Percentage given at the 1st *National Conference of Traditional Medicine and Complementary Practices* held in Luanda in August 2012.

chemical investigation. The plants were selected based on four criteria: the Use Report (UR), the correlation between traditional reported preparation and clinical data, the quality of the narrative content, and the novelty of the plant (Figure 4-1).

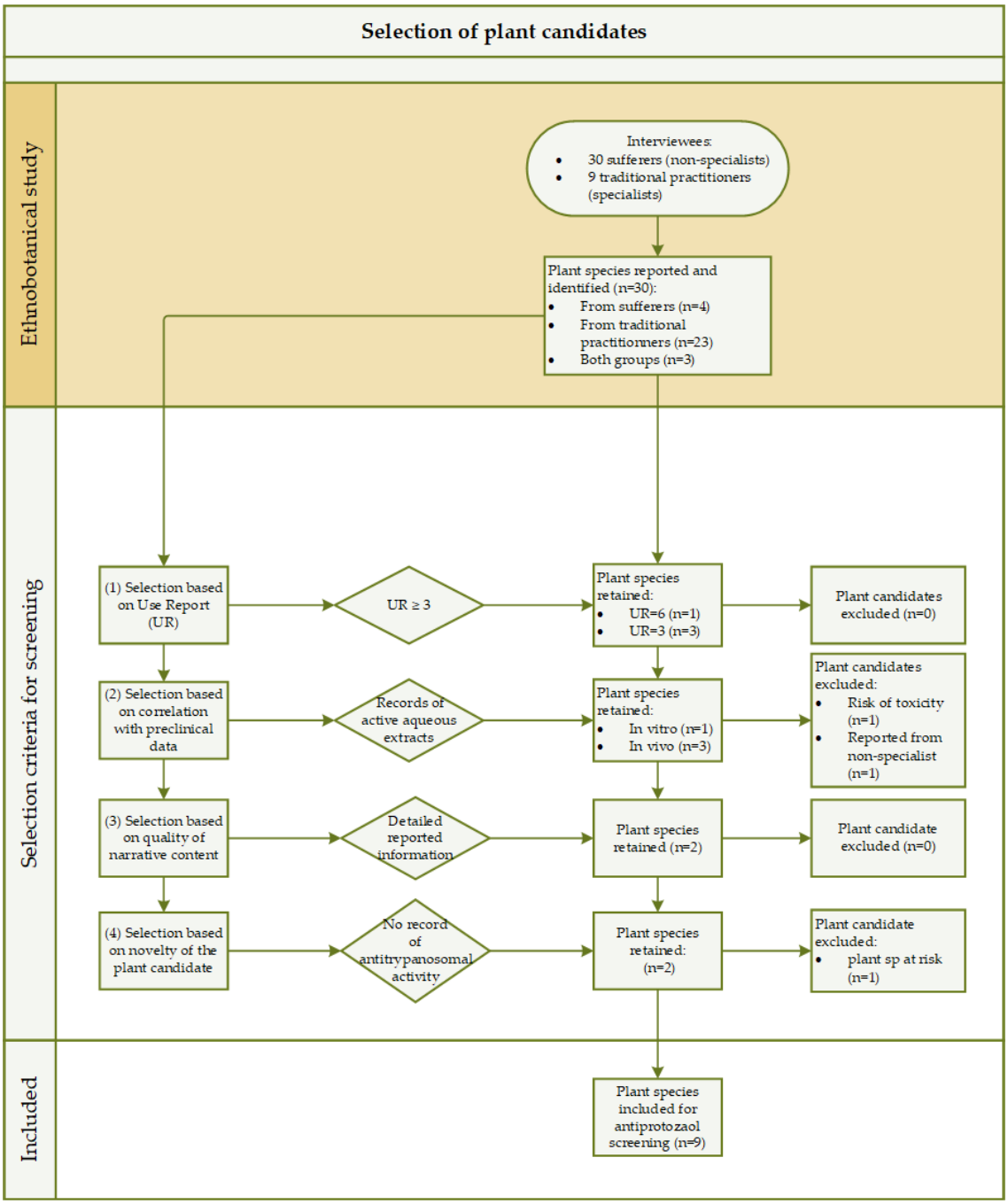


Figure 4-1: Flow diagram of the plant candidate selection process. Information in the horizontal corridor "ethnobotanical study" (highlighted in brown) arises from a previous ethnobotanical study run in Angola between 2017 and 2018, where 30 plant species had been identified [235]. Four inclusion criteria were applied (1) to (4). A total of 3 exclusion criteria were applied: potential risk of use (n = 1), reported from non-specialist informants (n = 1), and conservation status at risk (n = 1). Twelve plant species included: *Crossopteryx febrifuga, Vitex madiensis, Palisota schweinfurthii, Momordica charantia, Entada abyssinica, Sarcocephalus latifolius, Ocimum gratissimum, Securidaca longipedunculata, Nymphaea lotus, Brasenia schreberi, Brillantaisia owariensis and Daniellia alsteeniana. Three plant species excluded: Securidaca longipedunculata, Ocimum gratissimum and Daniellia alsteeniana.*

The selected plants are summarized in Table 4-1.

Table 4-1: Medicinal plants from Angola analyzed in this study. Collection number of the National Botanical Center in Luanda, Angola. n.d.: not determined.

Plant	Family	Collection number
Brillantaisia owariensis P.beauv.	Acanthaceae	7925
Brasenia schreberi J.F.Gmel	Cabombaceae	n.d.
Palisota schweinfurthii C.B.Clarke	Commelinaceae	894
Momordica charantia L.	Cucurbitaceae	8591
Entada abyssinica A.Rich.	Fabaceae	3468
Vitex madiensis Oliv.	Lamiaceae	7186
Nymphaea lotus L.	Nymphaeaceae	2513
Crossopteryx febrifuga (Afzel.ex G.Don) Benth	Rubiaceae	8212
Sarcocephalus latifolius (Sm.)E.A.Bruce	Rubiaceae	8231

4.3.2 Screening of extracts against *Trypanosoma brucei rhodesiense*

A total of 122 extracts were prepared from different parts of the nine plant species. Each plant part was extracted consecutively with hexane, dichloromethane, ethanol, methanol, and water. The extracts were tested for their in vitro growth inhibition (GI) activity against bloodstream forms of *Trypanosoma brucei rhodesiense* STIB900, our reference strain for drug testing against African trypanosomes at a concentration of 20 µg/mL. Of the 122 extracts, 16 showed a strong activity (GI of 91% - 100%), 13 extracts a marked activity (71% - 90% GI), 14 extracts a moderate activity (51% - 70% GI), 19 extracts a weak activity (31% - 50% GI) and 60 extracts were inactive (GI <30%). A detailed description of the plant species, the parts extracted, solvent, extraction yield, and percentage of growth inhibition (GI%) is given in Supplementary Table S1.

Only one of the nine investigated plants lacked inhibitory activity, *P. schweinfurthii*, whereas all other plants demonstrated at least one extract with a moderate antitrypanosomal activity. To the best of our knowledge, *B. schreberi* (Table S1, extracts ID 96, 98, 109, 110, 111) is reported for its antitrypanosomal activity for the first time here. Previous reports of *the in vitro* activity of *C. febrifuga* (leaves parts) [185], *S. latifolius* (roots parts) [146], *E. abyssinica* (root trunk and root bark) could be correlated to the obtained results. An *in vivo* study had provided promising results with a 70% methanol extract of *N. lotus*, reducing the parasitemia in mice infected with *T. b. brucei* at a dose of 100 mg/kg/day [180]. However, here the 70% methanol extract of *N. lotus* only showed a moderate *in vitro* inhibitory activity (Table S1, extract ID 89, 114). Furthermore, the methanolic extract of *V. madiensis* (leaves) and *M. charantia* (aerial parts) were found to be weakly active or inactive with a growth inhibition lying between 7%-35%, whereas two studies demonstrated an interesting *in vitro* activity [138, 185]. Such a variation among activity results could be attributed to a combination of genetic, environmental, physiological, and methodological factors influencing the chemical composition and bioactivity of the plant extracts from different varieties of the same species. The latter was reflected by the difference in inhibitory activity within the same extract type of

three different varieties of *N. lotus* collected from three different sites at different times (see Table S1, extracts IDs 88-94, 112-118, 110-121). Thus, *N. lotus* methanolic extracts IDs 89 and 114 exhibited a moderate activity, in contrast to the methanolic extract ID 121, which was inactive., 110-121, Supplementary material). Thus, *N. lotus* methanolic extracts IDs 89 and 114 exhibited a moderate activity in contrast to the methanolic extract ID 121, which was inactive.

15 active extracts from seven different species displayed a growth inhibition activity >70% at 20 µg/mL and were selected for further analysis (Table 4-2). Aside from activity, other considerations such as polarity and plant parts were also taken into account for the selection of the extracts. The selected extracts were also tested against two other trypanosomatid pathogens, *Trypanosoma cruzi* and *Leishmania donovani*, as well as the malaria parasite *Plasmodium falciparum*. In vitro 50% inhibitory concentrations (IC_{50}) and selectivity indices (SI) were determined (Table 4-3). In general, the extracts were more active against *T. b. rhodesiense* and *P. falciparum* than against *T. cruzi* and *L. donovani*. All the extracts had selectivity indices >1 for *T. b. rhodesiense* and *P. falciparum*. However, none of the extracts exhibited a high selectivity, which is not unusual due to the heterogeneous composition of the crude extracts. Further purification and isolation of the active constituents may highly improve the selectivity, as will also become apparent here (see Table 4-3).

Table 4-2: The 15 most promising extracts and their activity against *T. b. rhodesiense*. GI, growth inhibition; Ri, rhizomes; R, roots; AeP, aerial parts; Rb, root barks; L, leaves; EtOH, ethanol; MeOH, methanol; DCM, dichloromethane.

Extract ID	Plant name	Plant part	Solvent	GI (%) [1]
46	*E. abyssinica*	Ri	Aqueous	103
47	*E. abyssinica*	Ri	EtOH 80%	101
91	*N. lotus*	AeP	Hexane	98
54	*E. abyssinica*	Rb	EtOH 80%	98
109	*B. schreberi*	L	Aqueous	99
110	*B. schreberi*	L	EtOH 80%	96
111	*B. schreberi*	L	MeOH 70%	96
92	*N. lotus*	AeP	DCM	74
115	*N. lotus*	AeP	Hexane	96
116	*N. lotus*	AeP	DCM	81
69	*V. madiensis*	R	Hexane	79
20	*C. febrifuga*	L	Hexane	85
28	*V. madiensis*	L	Hexane	96
64	*M. charantia*	AeP	DCM	72
35	*B. owariensis*	L	Hexane	96

[1]measured at 20 µg/mL, mean of three independent replicates.

The aqueous and ethanol 80% extracts of *E. abyssinica* root (extracts IDs 46, 47, 54) showed antitrypanosomal activity and the aqueous extract (extract ID 46) exhibited the most potent IC_{50} value against *T. b. rhodesiense* with 1.8 µg/mL. This is in agreement with Freiburghaus et al. [148], who had demonstrated similar *in vitro* activity for the root methanolic extracts of *E.*

abyssinica harvested at two different periods (IC_{50} of 3.3 and 6.8 µg/mL vs. 4.1 µg/mL for ID 47, Table 4-3). Due to the several phytochemical studies already realized on this plant [184, 186-189], we concentrated our efforts on *B. schreberi* (extracts IDs 109, 110, 111) and *N. lotus* (extracts IDs 91, 92), which displayed IC_{50} values ≤10 µg/mL against *T. b. rhodesiense* and *P. falciparum* (Table 4-3) and whose antitrypanosomal activity had remained mostly unexplored.

Table 4-3: Antiprotozoal activities of the 15 selected active extracts, ranked by decreasing activity against T. b. rhodesiense. IC_{50} value and the selectivity index (SI), defined. Antitrypanosomal data and SI index represent the mean of three independent determinations and antiplasmodial data of two independent values. The IC_{50} values are in µg/mL. *L. donovani*: axenic amastigote. n.d: not determined. Ri = rhizomes; Rb = Root barks; AeP = aerial parts; L = leaves; Wp = leaves and stems.

Extract ID	Plant[plant part]	*T.b.rhodesiense*		*T. cruzi*		*L. donovani*		*P. falciparum*		L6
		IC_{50}	SI [1]	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	IC_{50}
46	*E. abyssinica*^{Ri}	1.8	4.5	14.0	0.6	29.9	0.3	6.5	1.2	6.3
47	*E. abyssinica*^{Ri}	4.1	4.0	16.1	1.0	43.4	0.4	12.7	1.3	16.3
91	*N. lotus*^{Li}	4.8	5.8	36.8	0.8	44.2	0.6	10.3	2.7	32.9
54	*E. abyssinica*^{Rb}	5.1	3.6	26.4	0.7	45.8	0.4	10.4	1.8	16.0
109	*B. schreberi*^{L}	5.9	2.9	26.7	0.6	53.0	0.3	3.5	4.9	33.8
110	*B. schreberi*^{L}	7.1	4.3	61.5	0.5	48.1	0.6	8.1	3.8	33.8
111	*B. schreberi*^{L}	7.9	4.0	65.9	0.5	42.4	0.7	7.5	4.2	36.0
92	*N. lotus*^{L}	9.8	3.8	56.7	0.7	14.5	2.5	6.3	5.9	42.4
115	*N. lotus*^{L}	11.9	2.5	45.5	0.6	20.1	1.5	14.7	2.0	34.5
116	*N. lotus*^{L}	12.2	3.6	56.3	0.8	17.7	2.5	7.9	5.5	49.7
69	*V. madiensis*^{R}	12.8	2.2	53.0	0.5	11.7	2.4	20.7	1.4	41.9
20	*C. febrifuga*^{L}	13.1	3.5	64.1	0.7	46.9	1.0	21.2	2.2	47.0
28	*V. madiensis*^{L}	13.6	1.7	42.2	0.6	23.2	1.0	23.9	1.0	22.8
64	*M. charantia*^{Wp}	30.5	1.1	48.1	0.7	25.5	1.3	8.7	3.9	26.0
35	*B. owariensis*^{L}	40.2	1.2	55.9	0.9	62.1	0.8	>50	n.d	48.2

[1] Selectivity Index, defined as the IC_{50} towards mammalian L6 cells divided by the IC50 towards the parasite.

B. schreberi is a floating-leaves plant originating from North America and distributed throughout Africa, Asia and Australia. It has so far not been investigated for its antitrypanosomal activity. *B. schreberi* is used in a traditional preparation in combination with *N. lotus* in the management of sleeping sickness in Angola. Both are aquatic plants, and the invasiveness of *B. schreberi* makes it a competitor to *N. lotus* in its natural environment see (Figure 4-5). Two studies investigated the antitrypanosomal activity in the Nymphaeaceae. The first is from Nigeria and reported antitrypanosomal activity of *Nymphaea odorata* with an IC_{50} value < 5µg/mL against *Trypanosoma brucei brucei* [238]. The second demonstrated that *N. lotus* had in-vivo antitrypanosomal potency [180]. However, no active molecules have been described so far from this plant.

We first selected two midrange polarity extracts for further chemical investigation: the ethanolic extract (extract ID 110, underlined in Table 4-3) of the leaves of *B. schreberi*[27] (IC_{50} = 7.1 ± 4.6 µg/L) and the dichloromethane extract (extract ID 116, underlined in Table 4-3) of

[27] It has to be clarified that in case of *B. schreberi* the leaves without petiole were extracted and tested, on the contrary to *N. lotus*, for which leaves and petiole were tested. In both cases, the plant part tested is "leaves".

the leaves and leaflets of *N. lotus* (IC_{50} = 12.2 ± 4.6 µg/mL). Then we used a semi-preparative chromatography–based bioactivity-guided fractionation to tentatively identify the active constituents.

4.3.3 Isolation of active constituents of *Brasenia schreberi* and *Nymphaea lotus*

The 80% ethanol crude extract of *B. schreberi* leaves (extract ID 110) was first submitted to vacuum liquid chromatography [VLC] to remove the highly polar constituents (Figure S1, Supplementary material). The VLC methanolic fraction (BS_EE80_VLC_MeOH) had demonstrated the most promising antitrypanosomal activity, with a GI value of 84.6% at 10 µg/mL (Figure S2, Supplementary material) and was selected for fractionation. To optimize the semi-preparative fractionation, the analytical conditions were first determined by HPLC and the conditions were then geometrically transferred to the semi-preparative HPLC with a gradient transfer method [239]. The fractions were pooled according to UV and ELSD peaks. (Figure 4-2, A and B). In total, 21 fractions were collected and assayed against *T. b. rhodesiense*. Finally, 5 fractions (F3, F6, F10, F11, F12) displayed a strong activity (GI% > 91% at 10 µg/mL), markedly stronger than the VLC methanolic extract itself (Figure 4-2C, Figure S2, Supplementary material).

Fractions F3 and F11 yielded two single compounds **1** and **6.** Fractions F6, F10, F12 were further purified using semi-preparative HPLC and yielded 5 minor compounds, **2 to 5** and **7** (Figure S3, Supplementary material). NMR and high resolution MS analysis resulted in identification of the seven active constituents, namely, gallic acid **(1)** [240]**,** methyl gallate **(2)** [240]**,** 2,3,4,6 tetragalloyl-glucopyranoside **(3)** [241]**,** ethyl gallate **(4)** [242]**,** 1,2,3,4,6 pentagalloyl-β-glucopyranoside **(5)** [243]**,** gossypetin-7-*O*-β-glucopyranoside **(6)** [244], hypolaetin-7-*O*-glucoside **(7)** [245] (Figure 4-3, Figures S4-11, Supplementary material).

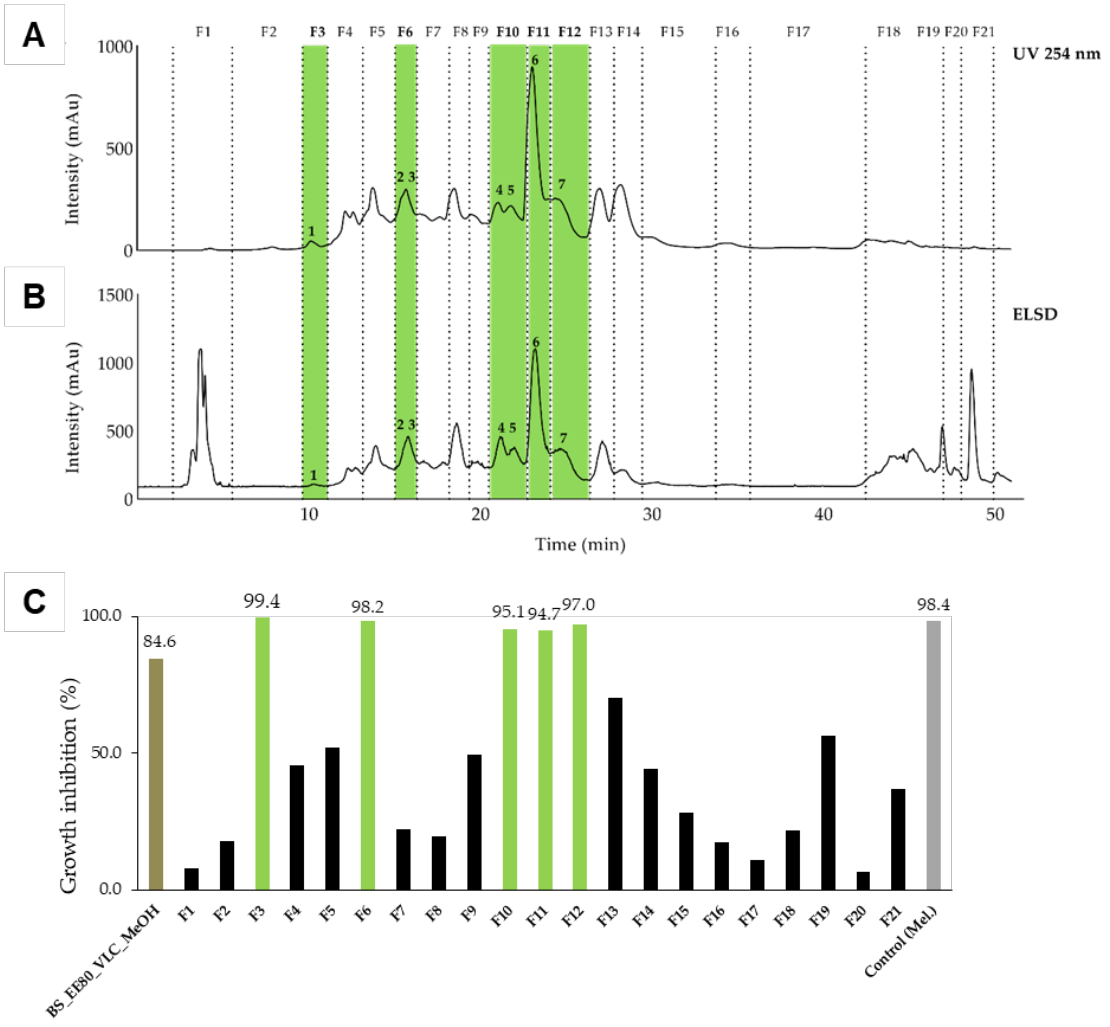


Figure 4-2 Semi-preparative HPLC chromatogram of the enriched methanolic extract of *B. schreberi* leaves with the collected fractions (F1 to F21) and the seven constituents 1 to 7. The separation of the components was detected by UV (A) and evaporative light scattering detectors (ELSD, B). The fractions were pooled according to UV and ELSD peaks. (C) Inhibitory activity of the VLC methanolic fractions against *T. b. rhodesiense* at 10 µg/mL. Five fractions (F3, F6, F10, F11, F12; green) displayed a strong activity (GI >91%). BS_EE80_VLC_MeOH: enriched VLC methanolic extract of the ethanolic extract of *B. schreberi* (80%). Control: melarsoprol at 0.072 µg/mL (Mel.)

Gallic acid **1** and its ester derivatives **2** to **5** are common natural polyphenols, widely present in plants and fungi. These secondary metabolites are known for a range of applications [246] and possess several activities such as antioxidant and neuroprotective [247], anti-inflammatory [248], anti-tumor [249-252], anti-bacterial [253-255]. Among the compounds studied, three **1**, **4**, and **7** have already been described from *B. schreberi* [256, 257] as well as gossypetin, the aglycone of compound **6.** This compound is predominantly present in the genus *Hibiscus* and has been isolated in many other plant species, like *Drosera peltata* [258] or *Equisetum fluviatile* [259].

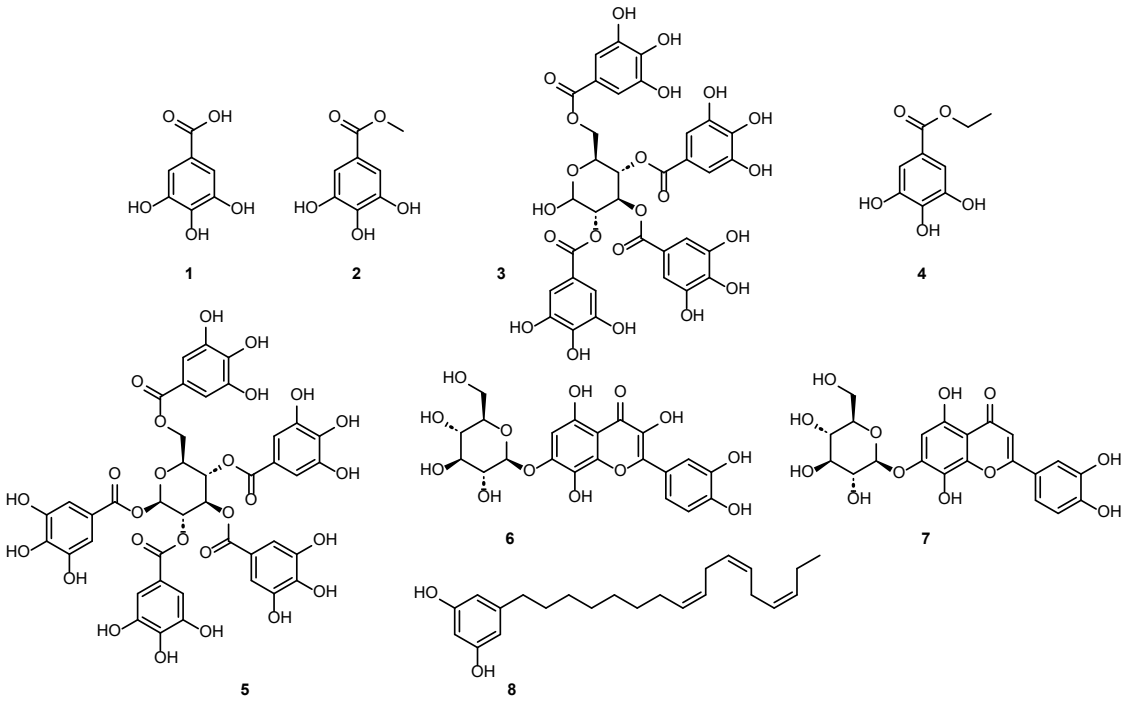


Figure 4-3: Structure of the identified compounds: galic acid **(1)**, methylgallate **(2)**, 2,3,4,6 tetragalloyl-glucopyranoside **(3)**, ethyl gallate **(4)**, 1,2,3,4,6 pentagalloyl-β-glucopyranoside **(5)**, gossypetin-7-O-β-glucopyranoside **(6)**, hypolaetin-7-O-glucoside **(7),** 5-[(8Z,11Z,14Z)-heptadeca-8,11,14-trienyl] resorcinol **(8)**.

However, the presence of compounds **2**, **3**, **4** and **6** in the genus *Brasenia* is reported for the first time here.

The dichloromethane extract of the leaves and leaflets of *N. lotus* (extract ID 116) was fractionated by normal phase semi-preparative chromatography using the same method as described previously but in normal phase. The 62 fractions generated were combined according to their UV and ELSD peaks (Figure 4-4, A and B) in eight fractions (F1-F8) and assayed against *T. b. rhodesiense* (Figure 4-4C). One active fraction (F4) demonstrated a strong activity (GI% 97.4% at 10 µg/mL). Analysis of fraction F4 revealed a single constituent structurally elucidated by NMR and high-resolution MS, and identified as a known alkenyl resorcinol **(8)** [260] (Figure 4-2). The resorcinolic lipids have been associated with plants, bacteria and fungi [261]. They are mostly found in the members of families *Anacardiaceae* (*e.g.* cashew, mango), *Ginkgoaceae* (*e.g.* Ginkgo biloba) and *Graminaceae* (*e.g.* cereals) [262]; to the best of our knowledge, occurrence in the *Nymphaeaceae* is reported here for the first time. *Nymphaea odorata* was described in a study on Nigerian medicinal plant for its activity against *Trypanosoma brucei brucei* [238]. The structures of the resorcinol **8** identified from *N. lotus* and the seven active constituents identified from *B. schreberi* **1-7** are shown in Figure 4-3.

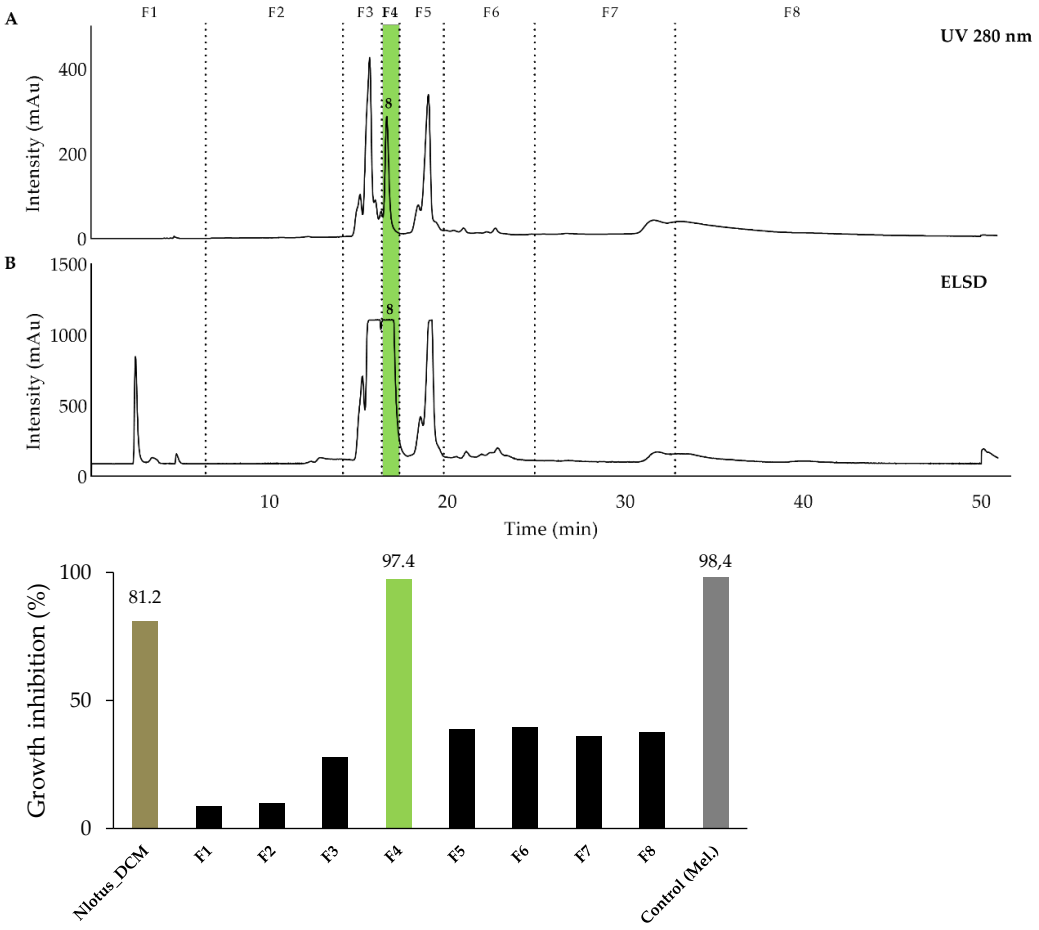


Figure 4-4: Semi-preparative HPLC chromatogram of the dichloromethane extract of *N. lotus* highlighting the collected fractions F1 to F8 and the active constituent 8. The separation of the components was detected by UV (A) and evaporative light scattering detectors (ELSD, B). (C) Inhibitory activity against T. b. rhodesiense at 10 µg/mL of the fractions. Only one fraction (F4, green) displayed a strong activity (97%). Legend: Nlotus_DCM: dichloromethane extract of N. lotus. Control: melarsoprol (Mel.) at 0.072 µg/mL.

4.3.4 Antiprotozoal activity of identified components

Except compound **3**, for which we had insufficient plant material, the identified compounds were evaluated against *T. b. rhodesiense* and other protozoa. Compounds **1, 2, 4,** and **5** could be purchased and were assayed against *T.cruzi*, *L. donovani* and *P. falciparum* (Table 4-4). Compounds **6, 7** and **8** were assayed only against *T. cruzi* and *L. donovani* (Table 4-3). Ethyl gallate **4** and methyl gallate **2** had IC_{50} against *T. b. rhodesiense* of 0.6 µg/ml and 1.1 µg/ml, respectively, as well as of 2.1 µg/mL and of 3.0 µg/mL against *P. falciparum*. The highest antitrypanosomal activity was found for gallic acid **1** and ethyl gallate **4** with IC_{50} against *T. b. rhodesiense* of 0.5 µg/mL and 0.6 µg/mL, respectively. None of the compounds demonstrated promising activity against *T. cruzi*. Resorcinol alkyl **8** had an IC_{50} of 2.5 µg/mL against *L. donovani* and a moderate selectivity (SI: 5.2). The two glycosidic flavones **6** and **7** displayed similar activities across the three trypanosomatides. The glucuronate flavones were less potent than their aglycones [263], suggesting that the antitrypanosomal activity of compounds

6 and **7** could be improved by removing the glycosidic part. The gallotannin pentagalloyl glucose **5** displayed the weakest overall antitprotozoal activity.

Our findings are in agreement with the reported activity of gallic acid **1** and ethyl gallate **4** against bloodstream forms of *T. b. brucei* [264, 265]. Gallic acid and its ester derivative **2** inhibited trypanosome the *sn*-glycerol-3-phosphate oxidase system of *T. b. brucei* in vitro [266]. Another possible mechanism of action of gallic acid is via its capacity to chelate iron and deprive the parasite [267, 268]. Due to the amphiphilic nature of alkyl esters **2** and **4,** these compounds might disrupt the plasma membrane, leading to trypanosome death [269]. Yet another possible target is the trypanosome alternative oxidase TAO; intriguingly, *T. brucei spp*. aquaglyceroporin null mutants, which are resistant to the drugs melarsoprol and pentamidine, are at the same time hypersensitive to inhibitors of TAO, including octyl gallate and propyl gallate [270].

Table 4-4: Antiprotozoal activity of the active compounds identified from *N. lotus* (extract ID 116, Table 4-3) and *B. schreberi* (extract ID 110, Table 4-3). IC_{50} values are in µg/mL and represent the mean of two independent experiments. *L. donovani*: axenic amastigote**.**

	T.b.rhodesiense		*T. cruzi*		*L. donovani*		*P. falciparum*		L6
	IC_{50}	**SI**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**	**SI**	**IC_{50}**
Gallic acid (**1**)	0.5	34	66	0.2	56	0.3	>10	n.d.	16
Methyl gallate (**2**)	1.1	15	16	1.0	8.5	1.9	2.1	7.8	16
Ethyl gallate (**4**)	0.6	25	16	0.9	6.8	2.2	3.0	4.9	15
Pentagalloyl-β-glucopyranoside (**5**)	20.0	1.0	44	0.5	15	1.4	6.7	3.1	21
Gossypetin-7-*O*-β-glucopyranoside (**6**)	5.5	1.6	12	0.8	53	0.2	n.d.	n.d.	8.9
Hypolaetin-7-*O*-glucoside (**7**)	5.7	3.2	49	0.4	52	0.4	n.d.	n.d.	19
Resorcinol-alkyl (**8**)	5.3	2.5	9.1	1.4	2.5	5.2	n.d.	n.d.	13

The results with amastigotes *T. cruzi* are consistent with the previous finding that galic acid and two of its ester derivatives **2** and **4** were inactive (IC_{50} > 100µM) against epimastigote forms of *T. cruzi* [271]. The detected antiplasmodial activity of compounds **2** and **4** were higher than previously reported [272]. However, another study had demonstrated a strong *in vitro* activity against *P. falciparum* for methyl gallate **2** (IC_{50} of 2.5 ng/mL) isolated from *Alectryon serratus* leaves [273] and an IC_{50} of 1.3 ng/mL for gallic acid **1**. The finding that methyl gallate **2** and ethyl gallate **4** have a higher antiplasmodial activity than gallic acid **1** itself is corroborated by a previous report [274]. The gallotannin pentagaloylglucose **5** has demonstrated several biological activities [275]. The antileishmanial activity obtained here was however lower than in a previous report [276]. The resorcinol alkyl **8** displayed an encouraging inhibitory activity when tested against axenic amastigotes *L. donovani* (IC_{50} =2.5 µg/mL). However, it did not demonstrate conclusive activity when tested in an intramacrophage assay (IC_{50} > 11 µg/mL). Interestingly, an isomer of compound **8**, the 5-heptadeca-8'Z,11'Z, 16-trienylresorcinol, was isolated from the mushroom *Merulius incarnatus* and had a similar activity against leishmania (IC_{50} =3.6 µg/mL) [277] as found here. The saturation degree of the alkyl chain impacts the bioactivity, resulting in loss of activity when

saturated [277] as well as the stereochemical orientation of the double bond system. In addition to the presence of unsaturation in the alkyl chain, a free phenolic hydroxyl group is required for bioactivity of resorcinol alkyls [278, 279]. Besides its inter-esting activity against leishmania, compound **8** displayed the best activity against *T. cruzi* among the isolated constituents (IC50 of 9.1 µg/mL). This is in agreement with Matutino Bastos et al. (2019), who had assayed two derivates of cardol against *T. cruzi* trypomastigote and amastigote forms [280]. Our results, together with these previous findings, ask for further investigation on resorcinol alkyls as potential compounds against *L. donovani* and *T. cruzi*.

4.3.5 Active constituents in local herbal preparation

In the northern province Uíge of Angola, the ethnotaxon "Longa dia simbi", is used for the treatment of sleeping sickness in the form of a decoction. "Longa dia simbi" in the local language Kikongo means "a tray", referring to the leaves lying as a tray on the surface of the water. "Longa dia simbi" is made of *Brasenia schreberi* and *Nymphaea lotus*. The two species are considered by local traditional healers as the same plant: *B. schreberi* as "female" and *N. lotus* as "male" (Figure 4-5). To validate the potential antitrypanosomal activity of the traditional preparation, the crude extracts of the decoction of *B. schreberi* (leaves) and of *N. lotus* (leaves and leaflets) were analyze by Ultra High-Performance Liquid Chromatography (UHPLC-MS) to detect the presence of the previously identified active constituents.

Figure 4-5: *Brasenia schreberi* (left) and *Nymphaea lotus* (right) in their natural environment in Angola, province of Uíge. Insets show the leaves (B, *B. schreberi* left and and *N. lotus* right) and flowers (A, *B. schreberi* "female"; C, *N. lotus* "male"). The two species are collected together, prepared as a decoction, and administered in the management of sleeping sickness.

Resorcinol alkyl, the major active component of *N. lotus*, was not detected in the decoction (extract ID 112, Table S1, Supplementary material), which was to be expected given the lipophilic structure of this compound. Nevertheless, the presence of compounds **1**, **4**, **6** and **7** was confirmed by LC-MS and UV analysis. These four constituents can account for the observed in vitro activity (GI% value 31-50%; Table S1, Supplementary material) of the aqueous extract of *N. lotus* (ID 112) against *T. b. rhodensiense*. The combined UV-MS detection and analysis of *B. schreberi* decoction (extract ID 109) revealed the presence of five active components **1**, **2**, **5**, **6** and **7**. These findings confirm the first activity screening, where the decoction extract of the

leaves of *B. schreberi* (ID 109) displayed a strong inhibitory activity against *T. b. rhodesiense* (GI% value >91% / see Table S1). A quantification of the identified active compounds **1**, **2**, **4**, and **5** was realized by UHPLC-UV Single Quadrupole MS analysis using pure reference substance (Table 4-5). These findings confirm the first activity screening, where the decoction extract of the leaves of *B. schreberi* (ID 109) displayed a strong inhibitory activity against *T. b. rhodesiense* (GI% value > 91% / see Table S1, Supplementary material). A quantification of the identified active compounds **1**, **2**, **4**, and **5** was realized by UHPLC-UV Single Quadrupole MS analysis using pure reference substance (see Table 4-5).

Table 4-5: Quantification of the main constituents in the decoctions of *B. schreberi* and of *N. lotus*. The values are in relation to the dried raw plant material (mg/g) and to the dry extract (mg/g).

Active component	*B. schreberi* decoction		*N. lotus* decoction	
	Raw material	Extract	Raw material	Extract
Gallic acid (**1**)	8.8	50	5.6	22
Methyl gallate (**2**)	0.007	0.04	0.005	0.022
Ethyl gallate (**4**)	n.d.	<19 ppm	n.d.	<19 ppm
Pentagalloyl-β-glucopyranoside (**5**)	0.39	2.3	0.09	0.36

4.4 Material and Methods

4.4.1 Chemicals

LC/MS grade acetonitrile was obtained from VWR International, (Dietikon, Switzerland) , and formic acid (99%) via Thommen-Furler AG (Rüti b. Büren, Switzerland) from Carlo Erba Reagents. Ultrapure water was obtained from an in-house ultrapure water system from Sartorius AG. The reference compounds gallic acid **1**, methyl gallate **2**, and ethyl gallate **4** were purchased from Sigma-Aldrich; 1,2,3,4,6-pentagalloyl-β-glycopyranoside **5** was obtained via Lucerna-Chem from MedChem Express. The reference compounds used as positive controls for drug efficacy testing were melarsoprol (Arsobal Sanofi-Aventis, received from WHO, Geneva, Switzerland), benznidazole (Epichem Pty Ltd, received from DNDi, Geneva, Switzerland), miltefosine (Sigma Aldrich, Buchs, Switzerland), chloroquine (Sigma Aldrich, Buchs, Switzerland), artesunate (Mepha Pharma AG, Aesch, Switzerland), and podophyllotoxin (Sigma Aldrich, Buchs, Switzerland).

4.4.2 Selection of the candidate plants

In an ethnobotanical study led by the authors over a 5-month period, from October 2016 to March 2017 in endemic regions of Angola, 30 plant species used in the management of sleeping sickness had been identified [235]. The data gathered through the ethnobotanical study together with a literature review on antitrypanosomal activity of the candidate plants provided information as pre-screen to select the plants for further pharmacological investigations. A total of 9 species were selected according to four inclusion criteria and three exclusion criteria (see Figure 4-1).

The four inclusion criteria were (1) the Use Report (UR), (2) the correlation be-tween traditional reported preparation and clinical data, (3) the quality of the narrative content, and

(4) the novelty of the plant. Briefly, the UR enabled quantifying the importance of the reported plant species among the interviewees. A higher UR value indicates that a plant species is more frequently mentioned within the informants, suggesting its greater importance or relevance to the cultural practices. Plant candidates with a UR ≥ 3 were retained (*n* = 4, namely *Crossopteryx febrifuga* (Afzel.ex G.Don) Benth, *Vitex madiensis* Oliv., *Palisota schweinfurthii* C.B.Clarke, and *Momordica charantia* L.). Based on a correlation between the traditionally reported herbal preparation (most often a decoction) and the preclinical results (in vitro and in vivo studies) of the extracts that mimicked most closely the traditional preparation (aqueous extracts), four plant species were included (*n* = 4, namely *Entada abyssinica* A.Rich., *Sarcocephalus latifolius* (Sm.) E.A.Bruce, *Ocimum gratissimum* L., and *Securidaca longipedunculata* Fresen). The quality of the narrative content referred to a qualitative appreciation of the information entrusted during the interview with the informant such as (a) the level of a detailed information, (b) a same response to a repeated question, (c) the veracity of the facts and (d) a collaborative attitude. Based on this parameter, one species was retained (*n* = 1, namely *Nymphaea lotus* L.). However, as explained under 4.3.5, *N. lotus* relates to the ethnotaxon "Longa dia simbi" that referred to two plant species, *N. lotus* and *B. schreberi* J.F. Gmel. Therefore, two plant candidates had to be considered for this third inclusion criteria (*n* = 2, namely *Nymphaea lotus* L. and *Brasenia schreberi* J.F. Gmel). The novelty criterion included plants species with an UR = 2 that had not been so far investigated for their antitrypanosomal activity (*n* = 2, namely *Brillantaisia owariensis* P.beauv. and *Daniellia alsteeniana* P.A.Duvign).

Three exclusion criteria were applied: (a) plant species known for their potential risk of use (*n* = 1, namely *Securidaca longipedunculata* Fresen.), (b) plant candidates reported from non-specialist informants (sufferers of trypanosomiasis) (*n* = 1 namely *Ocimum gratissimum* L.) or (c) species with a conservation status at risk (*n* = 1 namely *Daniellia alsteeniana* P.A.Duvign) [281].

4.4.3 Plant collection, identification and exportation

The plant material has been collected in the northern province Uíge of Angola. The nine plant species were authenticated by the Center of Studies and Scientific Investigation on Botanic of the Faculty of Science from University of Agostinho Neto, Luanda, Angola. The corresponding voucher specimen were deposited at the herbarium of the Center of Studies and Scientific Investigation on Botanic (Table 4-1).

Nagoya clearance was obtained by establishing a "Mutually Agreed Terms" (MAT) between the involved Parties. The MAT granted in the framework of this collaboration in July 2018, was the first official MAT of Angola, as has been notified in the Interim National Report on the Implementation of the Nagoya Protocol published on May 2019 on the platform of the ABS-Clearing House (https://absch.cbd.int/countries/AO, accessed on 15 Februaray 2024).

4.4.4 Extract preparation

Different types of extraction procedures were carried out on the powdered dry material. A detailed description of plant species, parts extracted, solvents, drug solvent ratio, and extraction yields is given in Table S1, Supplementary material.

For increasing polarity extraction, the plant material was successively extracted for 18 ± 2 h at room temperature under constant stirring with hexane, dichloromethane (DCM), methanol (MeOH), and distilled water (H_2O). After filtration, the extracts were evaporated under vacuum (Büchi Rotavapor, Switzerland) and dried un-der nitrogen stream. The solvent-free extracts were stored at 4 °C until use.

To replicate traditional preparations, a 20-fold quantity of water in relation to plant material was used for the extraction and boiled for 15 min. The decoction was filtrated with a Büchner funnel under vacuum or with a filter paper (Macherey-Nagel). The filtrates (AqDec) were freeze-dried and stored at 4 °C until use. Additionally, a 10% ethanolic extract (MetT) was produced by maceration at room temperature for 2 h. Filtration and drying were performed similarly as for the decoction.

For alcoholic extraction, an 80% ethanol extract (EtOH80%) was prepared by adding a ten-fold quantity of solvent in relation to plant material and extracted at room temperature for 2 h under constant agitation. Extracts were filtered through a filter paper (Macherey-Nagel), concentrated on a rotavapor (Büchi, Switzerland) at 40 °C until 60 mbar, freeze-dried, and stored at 4 °C until use. In order to assess previ-ously referenced activity of some plant species, the extraction procedure was reproduced as published (MeOH70%, AqMac, MeOH80%).

4.4.5 General chromatographic procedures

NMR spectroscopic data were recorded on a Bruker Avance III HD 600 MHz NMR spectrometer equipped with a QCI 5 mm Cryoprobe and a SampleJet automated sample changer (Bruker BioSpin, Rheinstetten, Germany). Chemical shifts (δ) were measured in parts per million (ppm) using the CD3OD signal as internal standard for all spectra (δH 3.31; δC 49.0) and coupling constants (J) are reported in Hz. Complete assignment was performed based on two-dimensional experiments (COSY, NOESY, HSQC and HMBC). High resolution tandem mass spectrometry (HRMS/MS) data were obtained on a Q Exactive Focus quadrupole-orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) using heated electrospray ionization (HESI-II) in the posi-tive and negative modes. Reverse and normal phase analysis were performed on a high-performance liquid chromatography (HPLC) Agilent 1260 Infinity LC and Ag-ilent 1100 series system, respectively, both consisting of a degasser, a mixing pump, an autosampler, and a diode array detector (DAD) (Agilent Technologies, Santa Clara, USA) connected to an evaporative light scattering detector (ELSD) Sedex LT-ELSD 85 or ELSD Sedex 55 (Sedere, Alfortville, France) to detect non-UV absorbing compounds. Fractionation of the enriched ethanolic extract of *B. schreberi* (BS_EE80_VLC_MeOH) and the dichloromethane extract of N. lotus (NLotus_DCM) were performed on a semi-preparative HPLC equipment (Armen modular spot prep II, Saint-Avé, France) connected to a ELSD Sedex 55 (Sedere, Alfortville, France). Sub-fractions of the VLC_MeOH extracts of B*. schreberi* were purified with a Shimadzu system equipped with a LC-20 A module pumps, an SPD-20 A UV/VIS detector, a 7725I Rheodyne® valve and an FRC-10 A fraction collector (Shimadzu, Kyoto, Japan).

4.4.6 Fractionation and isolation of active constituents

The 80% ethanolic extract of *B. schreberi* leaves and the dichloromethane extract of *N. lotus* leaves were fractionated and purified in order to isolate eight active constituents. The

ethanolic extract of *B. schreberi* was first subjected to a vacuum liquid chromatography (VLC) to remove very polar compounds. A 500 mL sintered-glass Büchner funnel attached to a vacuum line was packed with a C18 reverse phase Zeoprep® 40-63 µm (Lobar® Merck, Darmstadt, Germany), activated with methanol (4 x 250 mL) and equilibrated with distilled water (4 x 250 mL). The dry load composed of 3.53 g of the grinded extract mixed with the same stationary phase (1:1 w/w) was then loaded uniformly on the top of the stationary phase. The sample was eluted using water (6 x 250 mL) followed by methanol (6 x 250 mL) and washed with ethyl acetate (6 x 250 mL). The water fraction was lyophilized, while the methanol and ethyl acetate fractions were evaporated, to yield BS_EE80_VLC_H_2O (2.5 g), BS_EE80_VLC_MeOH (2.6 g) and BS_EE80_VLC_EtOAc (85 mg), respectively. The optimized analytical conditions for BS_EE80_VLC_MeOH were determined by HPLC as a step gradient from 5% to 14% of B in 5 min, then 14% to 30% of B in 5 min, 30% to 60% of B in 30 min and 60% to 100% of B in 5 min held during 10 min. Then, a geometrical gradient transfer was applied from analytical to semi-preparative scale using chromatographic calculations to ensure the same selectivity. The fractionation was performed on 80 mg of the extract (BS_EE80_VLC_MeOH) on a semi-preparative HPLC system (Armen modular spot prep II, Saint-Avé, France) using an Interchim C18 column (250 × 21.2 mm, 10 µm; In-terchim, Montluçon, France), with water (A) and methanol (B) containing 0.1% formic acid as mobile phase. The purification was performed using the same step gradient as the analytical conditions with a flow rate fixed at 17 mL/min. The UV detection was set at 254 nm and ELSD detection was performed under the following conditions: 40 °C, 3.1 bar N_2 and gain 8. The separation led to 61 fractions combined in 21 fractions according to their UV and ELSD signal (Fig. 4-1A, 4-1B). All fractions were evaporated and submitted to the in vitro growth inhibition assay (Fig. 4-1C). Fractions F3 and F11 exhibited an activity, and compounds 1 (0.7 mg) and 6 (4 mg) were identified as major compounds of these two fractions. The fractions F6, F10 and F12, which displayed an activity but could not be identified, were further purified on a Shimadzu semi-preparative equipment using a X-bridge C18 column (250 × 10 mm, 5 µm; Waters, Milford, MA, USA), with water (A) and methanol (B) containing both 0.1% formic acid as mobile phase. The purification of F6, F10 and F12 was performed using a step gradient from 17% to 25% of B in 60 min, held during 10 min. Briefly, F6 (5.4 mg), F10 (6.5 mg) and F12 (3.6 mg) were dissolved separately in 300 µL of methanol, added to a spatula of Zeoprep C18 silica (40-63 µm) and dried gently under N_2 stream. The mixture was loaded in a cartridge for dry load injection according to the method developed by Queiroz et al. [239]. The flow rate was fixed at 5 mL/min. The UV detection was set at 254 nm (F12) and 280 nm (F6, F10). The separation led respectively to 27 sub-fractions for F6, 21 for F21and 12 for F12. Sub-fractions were combined according to their UV detections (Figure S3, Supplementary material). Using this approach, compound 2 (0.1 mg) and 3 (0.1 mg) from F6, compound 4 (0.1 mg) and 5 (0.5 mg) from F10, compound 7 (0.6 mg) from F12 were isolated.

The dichloromethane extract of *N. lotus* was fractionated on a semi-preparative system (Armen modular spot prep II, Saint-Avé, France) using an Interchim SIHP column (21.2 x 250 mm, 10 µm; Interchim, Montluçon, France) equipped with a Uni-versal Guard Selectivity (UGS) SI pre-column cartridge holder (3 x 6 mm i.d., 10 µm); with hexane (A) and ethyl acetate (B) as mobile

phase. The purification was performed using a linear gradient from 5% to 100% of B in 40 min, held during 10 min. The flow rate was fixed at 17 mL/min, the UV detection at 280 nm. This fractionation led to 62 fractions combined in 8 fractions according to their UV detection (Fig. 4-3A and B). Using this approach, compound 8 (1.6 mg) was isolated from F4. The fraction was evaporated and submitted to the in vitro growth inhibition assay (Fig. 4-3C).
The four isolated compounds 3, 6, 7, 8 tested for their antitrypanosomal activity had their identity confirmed by MS data and NMR spectra, which were in accordance with published data [241, 244, 245, 260].The purity of the compounds was estimated by 1H-NMR and found to be >80% in all cases.

4.4.7 UHPLC_HRMS / MS analysis

UHPLC-HRMS/MS analysis was performed for the active extracts and pure compounds using a Waters® Acquity UPLC system connected to a Q Exactive Focus mass spectrometer (Thermo Scientific, Bremen, Germany) with a heated electrospray ionization (HESI-II) in the positive and negative modes. The optimized HESI-II parameters were as follows: source voltage, 3.5 kV (pos), 3.8 kV (neg); sheath gas flow rate (N_2), 55 units; auxiliary gas flow rate, 15 units; spare gas flow rate, 3.0; capillary temperature, 275 °C (pos), 320 °C (neg); S-Lens RF Level, 45. The mass analyzer was calibrated using a mixture of caffeine, methionine-arginine-phenylalanine-alanine-acetate (MRFA), sodium dodecyl sulfate, sodium taurocholate and Ultramark 1621 in an acetonitrile/methanol/water solution containing 1% formic acid by direct injection. The data-dependent MS/MS events were performed on the four most intense ions detected in full scan MS (Top 3 experiment). The MS/MS isolation window width was 1 Da, and the normalized collision energy (NCE) was set to 35 units. In data-dependent MS/MS experiments, full scans were acquired at a resolution of 35 000 FWHM (at m/z 200) and MS/MS scans at 17 500 FWHM both with a maximum injection time of 50 ms. After being acquired in a MS/MS scan, parent ions were placed in a dynamic exclusion list for 2.0 sec. Separation was achieved on an Acquity BEH C18 column (2.1 × 50 mm; 1.7 µm; Waters, Milford, MA, USA) with water (A) and acetonitrile (B) as mobile phase. The temperatures in the autosampler and in the column oven were fixed at 25 and 40 °C, respectively. Separation was performed with a linear gradient from 5% to 95% of B in 7 min, held during 1 min and then 1 min isocratic step at 5% of B for column reconditioning. Injection volume was set at 2 µL, the flow rate was fixed at 0.6 mL/min. An Acquity UPLC photodiode array detector (PDA) was used to acquire PDA spectra, which were collected from 210 to 450 nm. In positive ion mode, the di-isooctyl phthalate $C_{24}H_{38}O_4$ [M + H]+ ion (*m/z* 391.28429) was used as an internal lock mass.

4.4.8 HPLC-DAD-ELSD analysis

The extracts of *B. schreberi* were analyzed by HPLC with DAD and ELSD detection on an Interchim C18 column (250 × 4.6 mm i.d., 10 µm; Interchim, Montluçon, France) equipped with a Nova-Pak® C18 pre-column cartridge holder (4 µm, 60 Å), using a mobile phase consisting of water (A) and methanol (B) containing both 0.1% formic acid; separation was performed with a linear gradient from 5% to 100% of B in 40 min, held during 5 min; flow rate: 1 mL/min;

injection volume: 10 µL. The samples were diluted in methanol at 10 mg/mL. The UV detection was recorded at 210, 254, 280 and 366 nm. ELSD conditions: 45 °C, 3.5 bar N_2 and gain 8.
The DCM extract of *N. lotus* was analyzed by normal phase HPLC with UV and ELSD detections on a Interchim SIHP column (250 x 4.6 mm, 10 µm; Interchim, Montluçon, France) equipped with a Universal Guard Selectivity (UGS) SI pre-column cartridge holder (3 x 6 mm i.d., 10 µm); using a mobile phase consisting of hexane (A) and ethyl acetate (B); separation was performed as described in the previous paragraph except that the samples were diluted in ethyl acetate.

4.4.9 NMR spectroscopic data

The recorded spectroscopic data were compared with the ones available in the literature to identify unambiguously compound **1** as gallic acid [240], **2** as methyl gallate [240], **3** as a mixture of 2 tetragalloylglucose [241], **4** as ethyl gallate [242], **5** as 1,2,3,4,6-pentagalloyl-β-glucopyranoside [243], **6** as gossypetin 7-*O*-glucopyranoside [244], **7** as hypolaetin-7-*O*-glucoside [245] and **8** as an alkenyl resorcinol [260].

4.4.10 Quantification of active pure compounds

The analysis was performed with an UHPLC-MS (UPLC with QDa detector, Waters) equipped with an Acquity column (BEH C18 2. 1 mm x 100 mm, 1.7 µm with the following parameters: mobile phase water: formic acid (1000:1 v/v) (A) and acetonitrile (B); flow rate to 0.3 mL/min; column temperature 35 °C; temperature of the sample chamber 15 °C; injection volume 5 µL. The gradient used was set at 1% during 2 min, then 1-5% in 1 min, then 5-15% in 9 min held during 1 min, followed by 5 min from 15 to 95% held during 2.5 min. The analysis was carried out with the QDa detector in negative mode. The cone voltage was set to -15 V, ESI Capillary: 0.81 kV and the capillary temperature to 600 °C. The quantification was done over their respective mass traces in SIR mode (selected ion recording): 169 Da (gallic acid), 183 Da (methyl gallate), 197 Da (ethyl gallate) and 469 Da (1,2,3,4,6 pentagalloyl-β-glucopyranoside). Gallic acid was quantified with a PDA detector at 270 nm. A standard curve was used for the quantification from 0.240 - 245.7 mg/L (R^2 = 0.9998). Methyl gallate, ethyl gallate and 1,2,3,4,6 pentagalloyl-β-glucopyranoside were quantified by UHPLC-MS with a QDa detector and standard curves were established at 0.004 - 0.132 mg/L (R^2 = 0.9978), 0.094 - 94.080 mg/L (R^2 = 0.9999), and 0.28 – 175 mg/L (R^2 = 0.9993) respectively. Each sample was filtered (0.2 µm) and prepared at 1 mg/mL in distilled water.

4.4.11 Antiprotozoal activity and cytotoxicity testing

Growth inhibition (GI) activity against *T. b. rhodesiense* STIB 900 was determined as follows: in a 96-well microtiter plate, 50 µL of a HMI-9 medium supplemented with 15% heat-inactived horse serum is added to each well. 10 µl of the plant extract stock solution was added to each well. Then 50 µl of bloodstream-form trypanosomes were added, adjusted with a cell counter (CASY, Schärfe System, Germany) to 4 $x10^4$ cells/ mL. Another 50 µl of HMI-9 medium supplemented with 15% heat-inactived horse serum was added to each well of the microtiter

plate. The final concentration of the tested extract was 20 µL / mL. The plate was incubated at 37°C under a 5% CO_2 atmosphere for 72 h. 10 µL of Alamar blue solution (12.5 mg resazurin dissolved in 100 mL distilled water) was added to each well and the plate incubated for another 2 to 4 h. Then, the plate was read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Corporation, Sunnyvale, CA, USA) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Fluorescence was expressed as a percentage of the untreated control. A GI > 91 % was considered as a strong inhibitory activity, between 71-90% a marked activity, between 51-70% a moderate activity, between 31-50% a weak activity, < 30% as not active. IC_{50} determination was performed in a similar way, but with serial dilutions of the plant extract (or pure compound) covering a range from 90 to 0.123 µg/mL. IC_{50} values were calculated by linear interpolation selecting values above and below the 50% inhibition mark.

In vitro growth inhibitory activity of the extracts and pure compounds against *T. cruzi* (intracellular amastigote forms in L6 rat myoblasts), *L. donovani* (axenic amastigote forms in acidic medium or intracellular amastigotes in mouse primary macrophages), and *P. falciparum* (erythrocytic stage in culture) was determined as described previously [282].
Testing against intracellular *L. donovani* was performed as follows: mouse peritoneal macrophages (4 x 104 in 100 µL RPMI 1640 medium with 10% heat-inactivated FBS) in 96-well plates were infected with amastigotes (2 x 105 in 100 µL medium). After 24 h, the medium was exchanged twice to remove free amastigotes. Test compounds were added, and the plates incubated for 96 h at 37 °C under 5% CO_2. Then, the medium was removed, the cells were fixed with 50 µl 4% formaldehyde, and stained with 5 µM DRAQ5. Nine images were taken per well on a ImageXpress XLS (MD) microscope using a 20x air objective (635 nm excitation: 690/50 emission) and analyzed with a script developed on Meta Xpress (MD). Cytotoxicity against L6 cells was assessed by using a similar protocol as outlined for IC_{50} determination with *T. b. rhodesiense*, except that rat skeletal myoblasts (L6 cells) were used. The medium was RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum. Reference compounds were melarsoprol for *T. b. rhodesiense*, benznidazole for *T. cruzi*, miltefosine for *L. donovani*, chloroquine and artesunate for *P. falciparum*, and podophyllotoxin for L6 cells.

4.5 Conclusion

Aiming to provide preliminary safety and efficacy validation of traditional herbal preparations, we investigated the cytotoxicity and antitrypanosomal activity of different extracts from medicinal plants that are being used in Angola in the treatment of sleeping sickness. After a preliminary activity screening, 15 active extracts were retained. Two extracts of two different aquatic plants, *Brasenia schreberi* and *Nymphaea lotus*, displayed IC_{50} values ≤ 10 µg/ml. Interestingly, these two Nymphaeales are being used in combination in a traditional preparation for the management of sleeping sickness in the northern part of Angola. While this is the first investigation of their antitrypanosomal constituents, *B. schreberi* and *N. lotus* have

been investigated for several other bioactivities such as antioxidant [283] and anti-inflammatory [256, 284], antibacterial [285-287], antialgal [288], anti-adipogenic [257], as well as cholesterol lowering [289] and inhibition of HIV-1 reverse transcriptase [290, 291].

In the present study, we report on the bioactivity-guided fractionation of the dichloromethane extract of *N. lotus* and the VLC methanolic extract of *B. schreberi* with the identification of 8 active constituents **1-8**. The presence of several antitrypanosomal compounds, gallic acid, methyl gallate, ethyl gallate and 1,2,3,4,6-pentagalloyl-β-glucopyranoside in the traditional preparation made of the leaves and leaflets of *B. schreberi* and *N. lotus* provides first evidence of the potential of the local preparation in the management of sleeping sickness in Angola. However, toxicity and in vivo efficacy remain to be further investigated.

Funding: We gratefully acknowledge financial support by the Swiss TPH and the R. Geigy-Stiftung. R.R. and P.M. were supported by the Swiss National Science Foundation (grant CRSII5_183536).

Acknowledgments: My first thanks go to the former Director of the FOMETRA in Uíge (Angola) Sr. António Kiakubua for his role as "gate opener" and his long-lasting support. Many thanks are also due to the late Coordinator of the CATEMATA, Sr. Kitoko Mayavângwa called Papa Kitoko, for helping me building trust with the traditional practitioners. Dr. Vasilisa Pedan from ZHAW for her sound advice in extract preparation and her constant availability as well as Katrin Jedrys for valuable comments and constant encouraging words and kindness. Special thanks to Sylvan Hälg from the Swiss TPH for his support in the in vitro assays. Robin Huber for his support in conducting the dry-load fractionation. A particular thank to Dr. Sergio Wittlin for giving the opportunity to test the antiplasmodial potential of the extracts.

Conflicts of Interest: The authors declare no conflict of interest.

4.6 Supplementary material

Table S1: Overview of all the plants extracts, their preparation and antitrypanosomal activity. Growth inhibition activity is categorized in five groups: strong activity in green (GI of 91% - 100%), marked activity in brown (71% - 90% GI), moderate activity in dark blue (51% - 70% GI), weak activity in light blue (31% - 50% GI) and inactive in greyish blue (GI <30%).

Extract ID	Plant name	Plant part	Sample labeling	Solvent	Raw mass of dried plant material (g)	Drug/ solvent ratio	Weight of dried extract (g)	Yield of extract (%)	Growth inhibition (%) at 20 µg/ml
1	*N. lotus & B. schreberi (mixture)*	leaves	11	AqDec	10.18	1:20	1.703	16.7	97
2			12	EtOH80%	15.20	1:10	2.27	14.9	95
3			13	MeOH70%	14.8	1:8	1.95	13.2	96
4	*C. febrifuga*	trunk bark	46	AqDec	30.09	1:20	3.794	12.6	17
5			47	EtOH80%	15.1	1:10	1.88	12.5	26
6			4a	Hexane	100.04	1:3	0.075	0.1	38
7			4b	DCM		1:3	0.088	0.1	31
8			4c	MeOH		1:3	9.139	9.1	26
9			4d	H20		1:3	1.479	1.5	17
10	*C. febrifuga*	root	58	AqDec	15.09	1:20	1.673	11.1	16
11			59	AqMac	15.1	1:20	1	6.6	18
12			510	EtOH80%	13.7	1:10	0.92	6.7	22
13			5a	Hexane	50.52	1:3	0.051	0.1	37
14			5b	DCM		1:3	0.080	0.2	37
15			5c	MeOH		1:3	4.576	9.1	31
16			5d	H20		1:3	0.589	1.2	12
17	*C. febrifuga*	leaves	611	AqDec	15.08	1:20	3.749	24.9	22
18			612	EtOH80%	15.114	1:10	2.4	15.9	28
19			613	MeOH80%	15.1	1:10	2.95	19.5	26
20			6a	Hexane	100	1:3	1.247	1.2	85
21			6b	DCM		1:3	0.571	0.6	56
22			6c	MeOH		1:3	11.191	11.2	21
23			6d	H20		1:3	2.656	2.7	12
24	*V. madiensis*	leaves	714	AqDec	15.0928	1:20	2.442	16.2	28
25			715	EtOH80%	15.2	1:10	1.82	12.0	33
26			716	MetT	15	1:20	2.656	17.7	29
27			717	MeOH	10.5	1:4.2	1.2	11.4	35
28			7a	Hexane	83.94	1:3	0.688	0.8	96
29			7b	DCM		1:3	0.746	0.9	62
30			7c	MeOH		1:3	12.191	14.5	22
31			7d	H20		1:3	2.667	3.2	17
32	*B. owariensis*	leaves	818	AqDec	19.995	1:20	5.864	29.3	10
33			819	EtOH80%	20.3	1:10	2.681	13.2	23
34			820	MetT	15.1	1:20	3.509	23.2	11
35			8a	Hexane	100.13	1:3	1.007	1.0	96
36			8b	DCM		1:3	1.621	1.6	74
37			8c	MeOH		1:3	5.123	5.1	34
38			8d	H20		1:3	8.353	8.3	22
39	*E. abyssinica*	trunk bark	1021	AqDec	30.259	1:20	5.539	18.3	61
40			1022	EtOH80%	50.19	1:10	3.4	6.8	70
41			10a	Hexane	100.18	1:8	0.404	0.4	75
42			10b	DCM		1:8	0.560	0.6	52
43			10c	MeOH		1:8	13.405	13.4	57
44			10d	H20		1:8	0.579	0.6	13

45	*E. abyssinica*	root inside	11a23	AqDec	15.01	1:20	1.431	9.5	85
46			11a24	AqMac	15	1:20	2.1	14.0	103
47			11a25	EtOH80%	20.123	1:10	2.133	10.6	101
48			11a1	Hexane	100.08	1:8	2.608	2.6	58
49			11a2	DCM		1:8	0.167	0.2	33
50			11a3	MeOH		1:8	12.731	12.7	35
51			11a4	H20		1:8	1.828	1.8	0
52	*E. abyssinica*	root bark	11b26	AqDec	20.329	1:20	4.349	21.4	88
53			11b27	AqMac	10.2	1:20	1.6	15.7	89
54			11b28	EtOH80%	15.121	1:10	2.249	14.9	98
55		root (all)	11b1	Hexane	100.08	1:4	0.395	0.4	20
56			11b2	DCM		1:4	0.317	0.3	53
57			11b3	MeOH		1:4	17.425	17.4	93
58			11b4	H20		1:4	0.574	0.6	29
59	*M. charantia*	whole plant	1229	AqDec	15.015	1:20	2.822	18.8	16
60			1230	AqMac	5	1:20	0.97	19.4	16
61			1231	EtOH80%	24.2	1:10	2.514	10.4	27
62			1232	MeOH80%	9.9	1:10	1.27	12.8	22
63			12a	Hexane	40.81	1:3	0.152	0.4	58
64			12b	DCM		1:3	0.588	1.4	72
65			12c	MeOH		1:3	1.52	3.7	30
66			$12d_1$	H20		1:3	3.949	9.7	22
67	*V. madiensis*	root	1333	AqDec	30.4	1:20	4.858	16.0	26
68			1334	EtOH80%	15.1	1:10	1.423	9.4	24
69			13a	Hexane	100.13	1:3	0.102	0.1	79
70			13b	DCM		1:3	0.069	0.1	50
71			13c	MeOH		1:3	7.818	7.8	7
72			13d	H20		1:3	3.174	3.2	0
73	*S. latifolius*	root	1435	AqDec	15.136	1:20	2.357	15.6	18
74			1436	AqMac	5	1:20	0.47	9.4	16
75			1437	EtOH80%	29.8	1:10	1.922	6.4	18
76			1438	MeOH	10.1	1:10	0.64	6.3	32
77			14a	Hexane	52.08	1:3	1.911	3.7	18
78			14b	DCM		1:3	0.264	0.5	60
79			14c	MeOH		1:3	2.866	5.5	36
80			$14d_1$	H20		1:3	1.285	2.5	24
81	*P. schweinfurthii*	leaves	1539	AqDec	15.7	1:20	2.178	13.9	10
82			1540	aqMac	5.3	1:20	0.91	17.2	8
83			1541*	EtOH80%	30	1:10	2.208	7.4	15
84			15a	Hexane	50.06	1:5	0.234	0.5	41
85			15b	DCM		1:5	0.387	0.8	17
86			15c	MeOH		1:5	1.085	2.2	13
87			$15d_1$	H20		1:5	5.373	10.7	6
88	*N. lotus*	whole plant	1642	AqDec	14.7	1:20	3.8	25.9	35
89			1643	MeOH70%	15.1	1:8	1.73	11.5	61
90			1644**	EtOH80%	15	1:10	2.196	14.6	58
91			16a	Hexane	21	1:3	0.616	2.9	98
92			16b	DCM		1:3	0.282	1.3	74
93			16c	MeOH		1:3	0.716	3.4	13
94			$16d_1$	H20		1:3	2.018	9.6	22
95	*B.schreberi*	whole plant	1745	AqDec	10.1	1:20	1.19	11.8	41
96			1746	EtOH80%	20.394	1:10	2.163	10.6	92
97			1747	MeOH70%	15	1:8	1.02	6.8	8
98			17a	Hexane	50.08	1:2	0.179	0.4	94
99			17b	DCM		1:2	0.096	0.2	79
100			17c	MeOH		1:2	1.954	3.9	76
101			$17d_1$	H20		1:2	1.585	3.2	16

102	*B.schreberi*	stem (sub aquatic part)	1848	AqDec	10.51	1:20	1.232	11.7	20
103			1849	EtOH80%	20.08	1:10	1.804	9.0	31
104			1850	MeOH70%	15.1	1:8	0.79	5.2	21
105			18a	Hexane	19.72	1:5	0.181	0.9	75
106			18b	DCM		1:5	0.079	0.4	70
107			18c	MeOH		1:5	1.645	8.3	23
108			$18d_1$	H20		1:5	1.225	6.2	27
109	*B. schreberi*	leaves	1951	AqDec	20	1:20	3.5	17.5	99
110			1952	EtOH80%	25.2	1:10	4.164	16.5	96
111			1953	MeOH70%	15.1	1:8	1.83	12.1	96
112	*N. lotus (bungo batch)*	whole plant	2054	AqDec	15.1	1:20	3.9	25.8	32
113			2055	EtOH80%	19.806	1:10	2.31	11.7	46
114			2056	MeOH70%	14.96	1:8	2.07	13.8	51
115			20a	Hexane	20.12	1:5	0.404	2.0	96
116			20b	DCM		1:5	0.199	1.0	81
117			20c	MeOH		1:5	1.719	8.5	23
118			$20d_1$	H20		1:5	1.544	7.7	9
119	*N. lotus (Damba batch)*	whole plant	2157	AqDec	15.1	1:20	3.5	23.2	7
120			2158*	EtOH80%	19.806	1:10	2.31	11.7	23
121			2159	MeOH70%	13.02	1:8	1.29	9.9	18
122	MIX	whole plant	Mix60	AqMac	5.08	1:20	2.33	15.4	6
		root			5.12				
		leaves			4.93				

Figure S1*:* ELSD chromatograms for the ethanolic extract of *B. schreberi* before and after VLC enrichment.

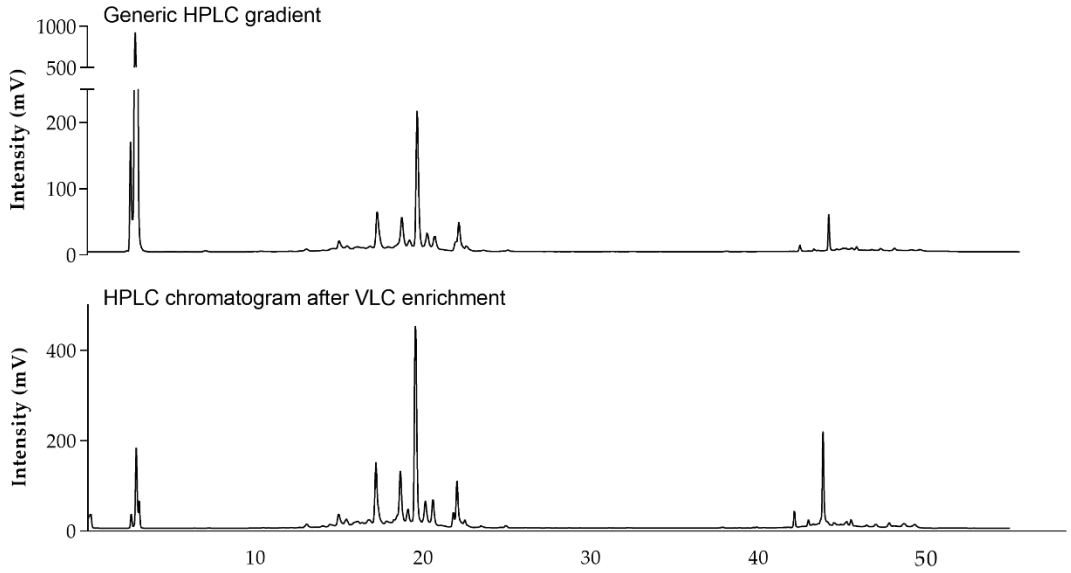


Figure S2: Growth inhibition activity (%) against *T. b. rhodesiense* of *B. schreberi* leave extracts at 20 and 10 µg/mL. The inhibitory activity of the crude ethanol 80% extract is compared to its enriched VLC fractions. VLC methanolic fraction (VLC_MeOH) displayed most promising antitrypanosomal activity with a GI (%) of 84.6 at 10 µg/mL and 95.1% at 20 µg/mL. Legends for *B. schreberi* leave extracts: ethanol 80% crude extract (BS_EE80), VLC aqueous fraction (BS_EE80_VLC_H2O), VLC methanolic fraction (BS_EE80_VLC_MeOH), VLC ethyl acetate fraction (BS_EE80_VLC_EtoAc).

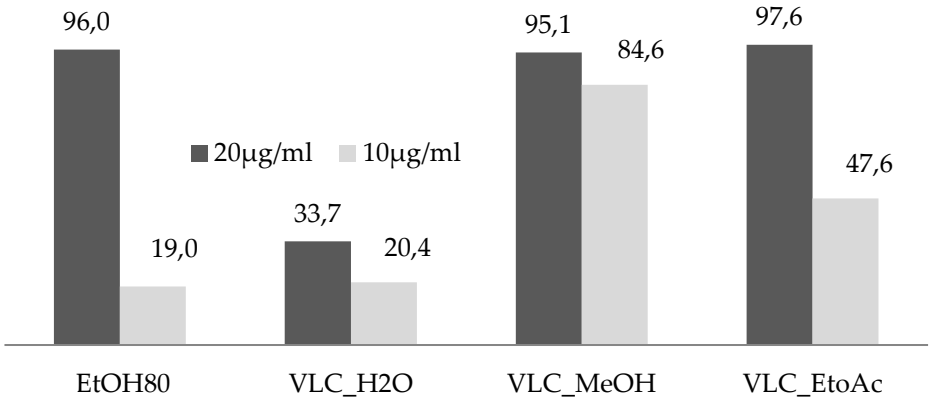
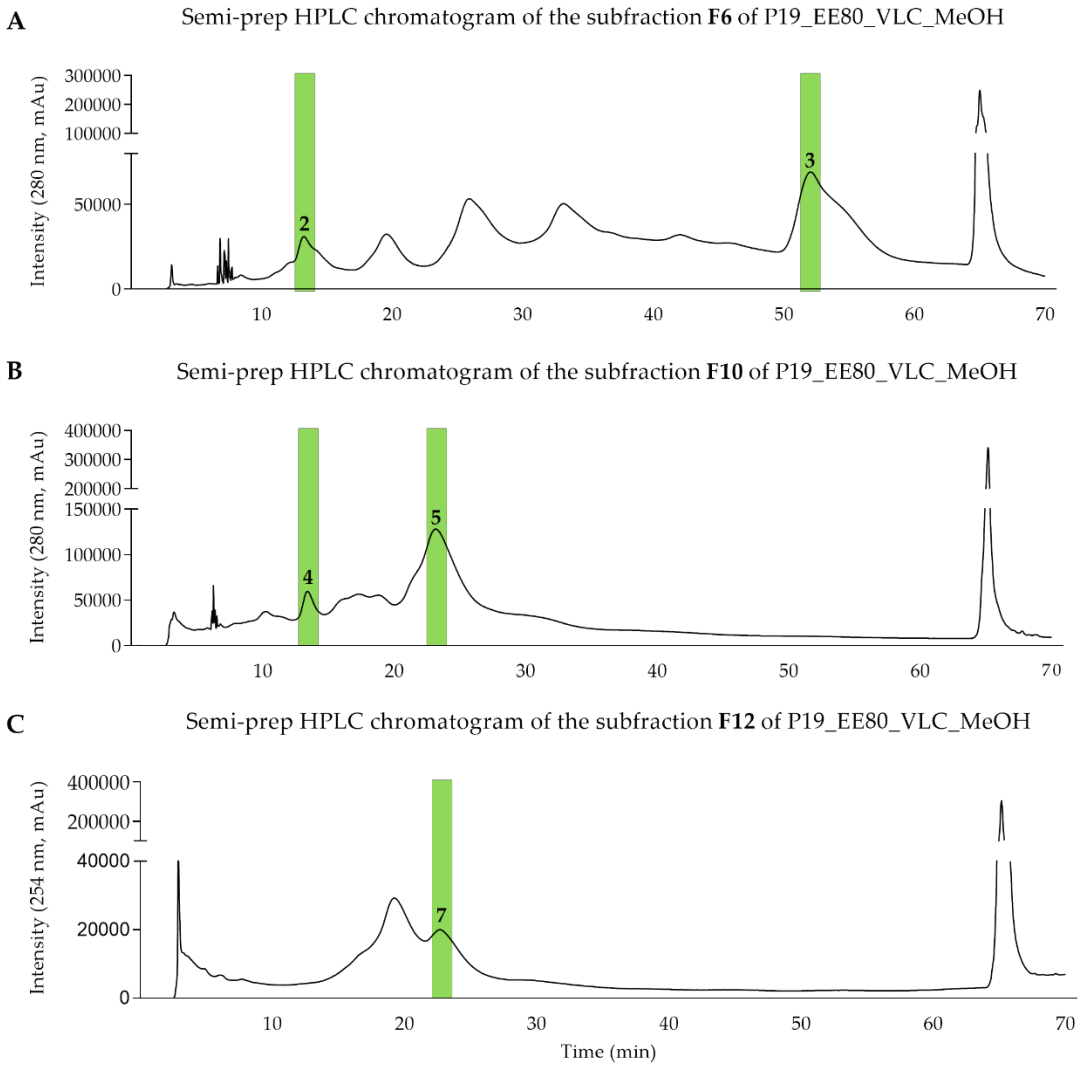
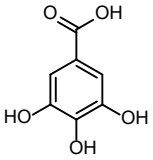


Figure S3: Separation of fraction F6 (chromatogram A), F10 (chromatogram B) and F12 (chromatogram C) of VLC methanolic extract of the leaves of *B. schreberi*. F6 yielded methyl gallate (2) and 2,3,4,6 tetragalloyl-glucopyranoside (3). UV detector at 280 nm. Subfractionation of F10 yielded ethyl gallate (4) and 1,2,3,4,6 pentagalloyl-β-glucopyranoside (5); UV detector at 280 nm. F12 yielded hypolaetin-7-O-glucoside (7); UV detector at 254 nm. P19_EE80_VLC_MeOH = VLC ethanolic extract of the leaves of *B. schreberi*.



NMR data

Figure S4: **^{1}H NMR data and spectrum of compound 1 in CD_3OD at 600 MHz**.
Gallic acid (**1**) [240]: ^{1}H NMR (CD_3OD, 600 MHz) δ 7.04 (2H, s, H-2, H-6). HRESIMS *m/z* 169.0136 [M-H]$^{-}$ (calcd for $C_7H_6O_5^{-}$, 169.01370, Δ = -3.7 ppm).



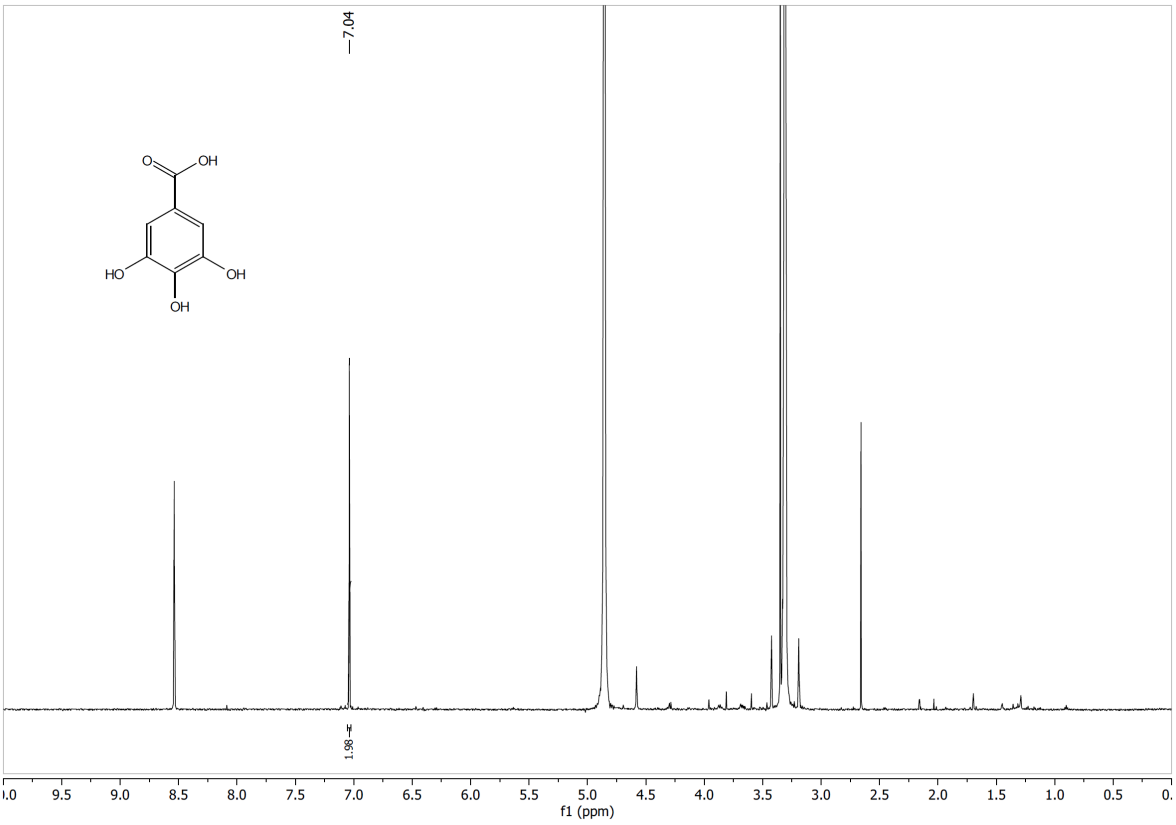
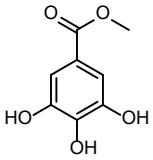
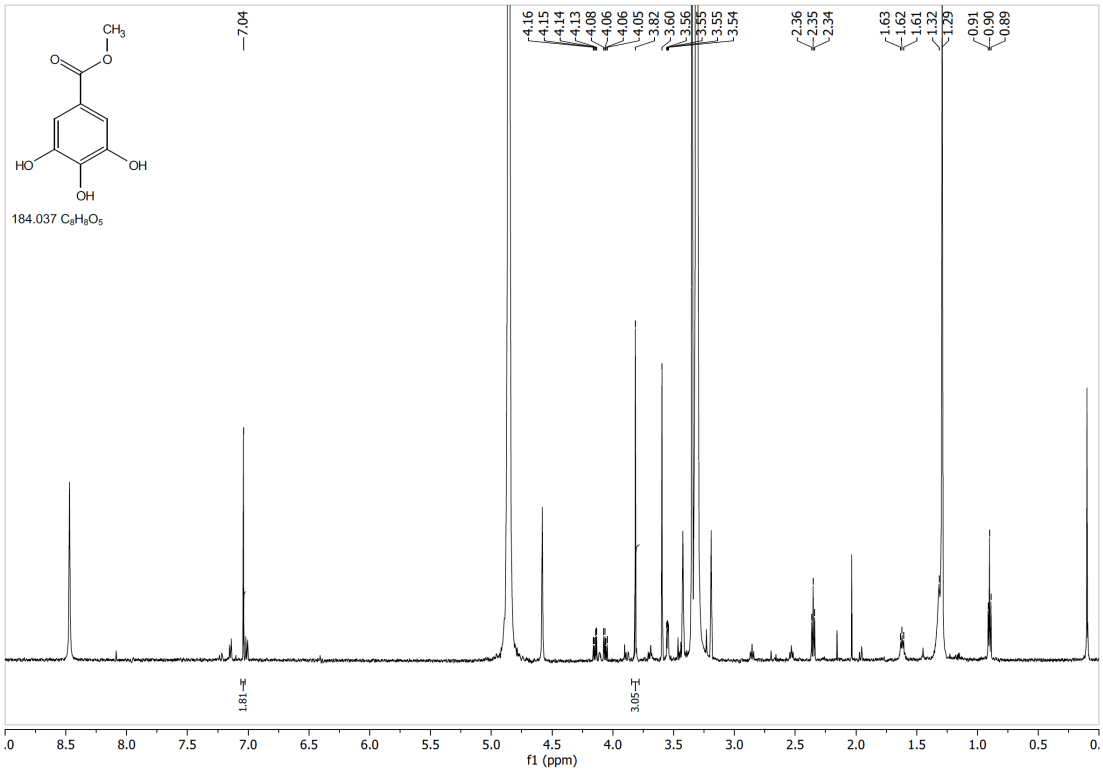
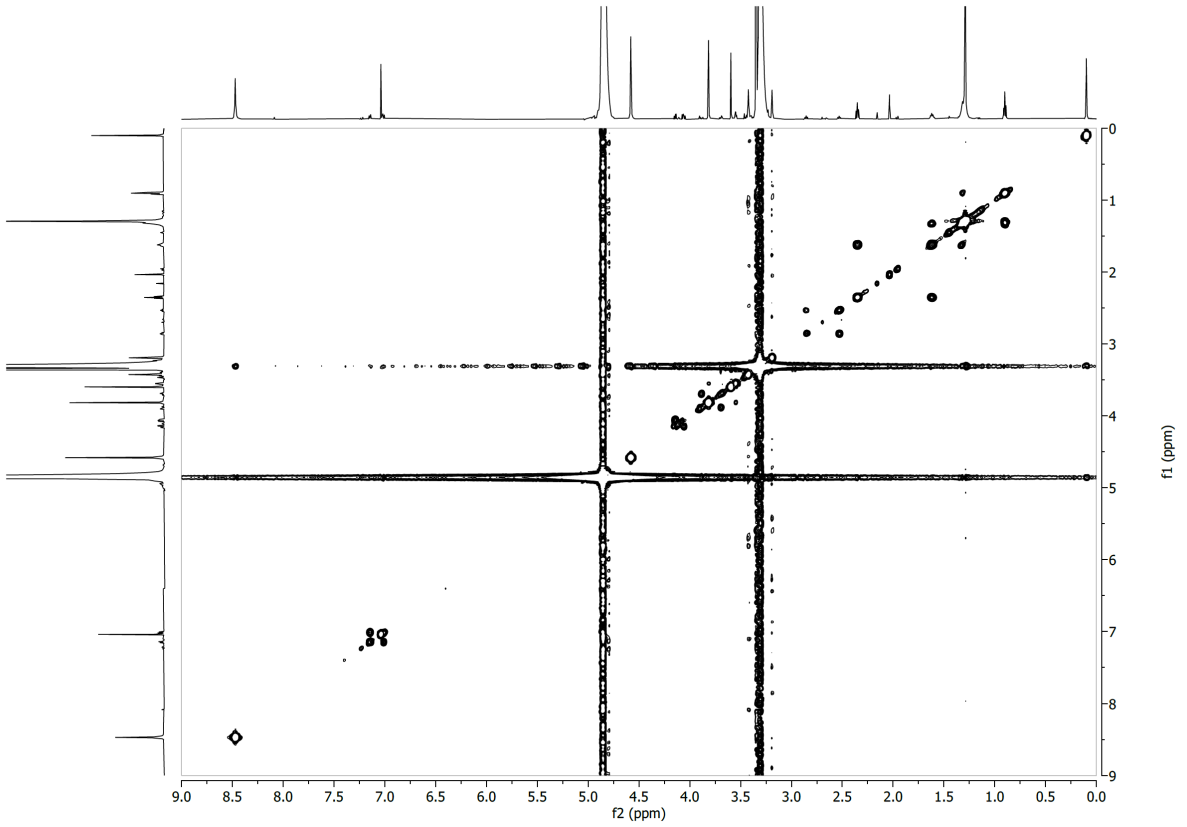


Figure S5: **NMR data and spectra of compound 2 in CD_3OD at 600 MHz.**
Methyl gallate (**2**) [240]: ^{1}H NMR (CD_3OD, 600 MHz) δ (3H, s, CH_3-8), 7.04 (2H, s, H-2, H-6); ^{13}C NMR (CD_3OD, 151 MHz) δ 51.9 (CH_3-8), 109.7 (C-2, C-6), 139.6 (C-4), 163.2 (C-3, C-5), 168.8 (C-7). HRESIMS m/z 183.0302 [M-H]- (calcd for $C_8H_7O_5^{-}$, 183.02935, Δ = 1.8 ppm).

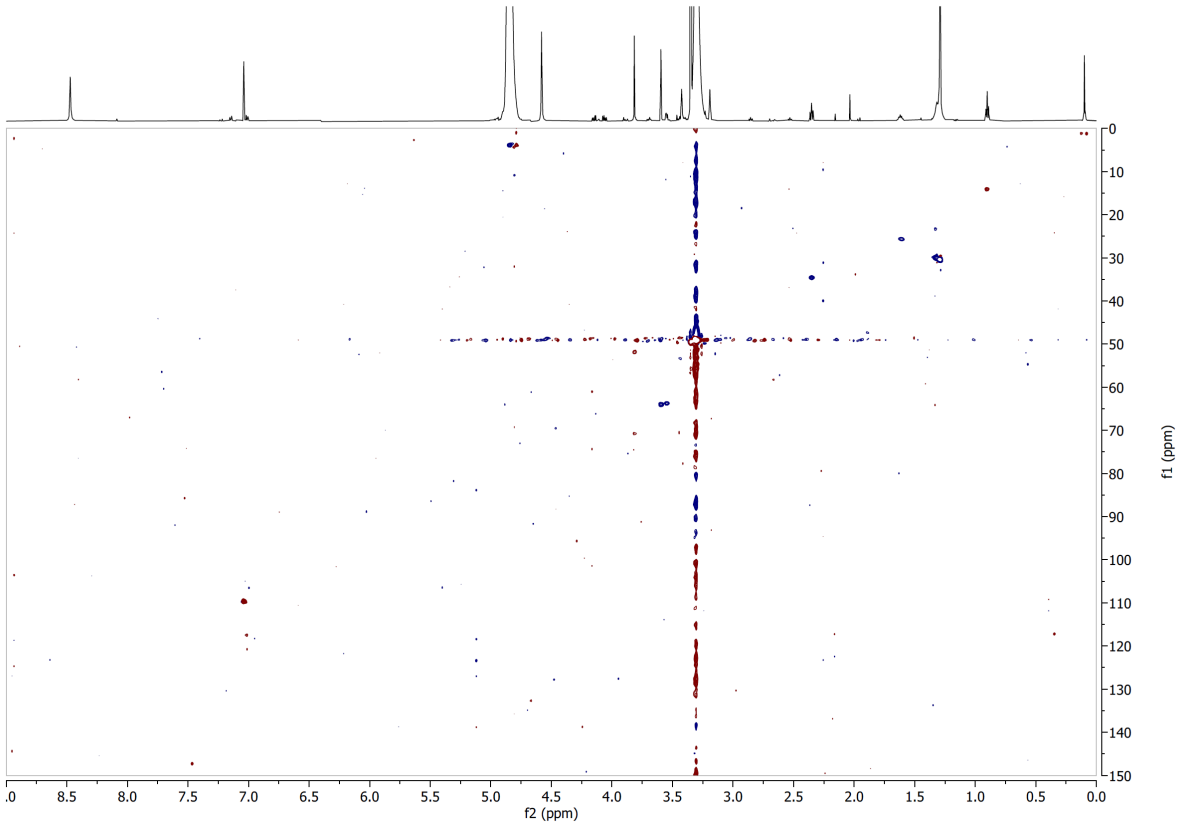




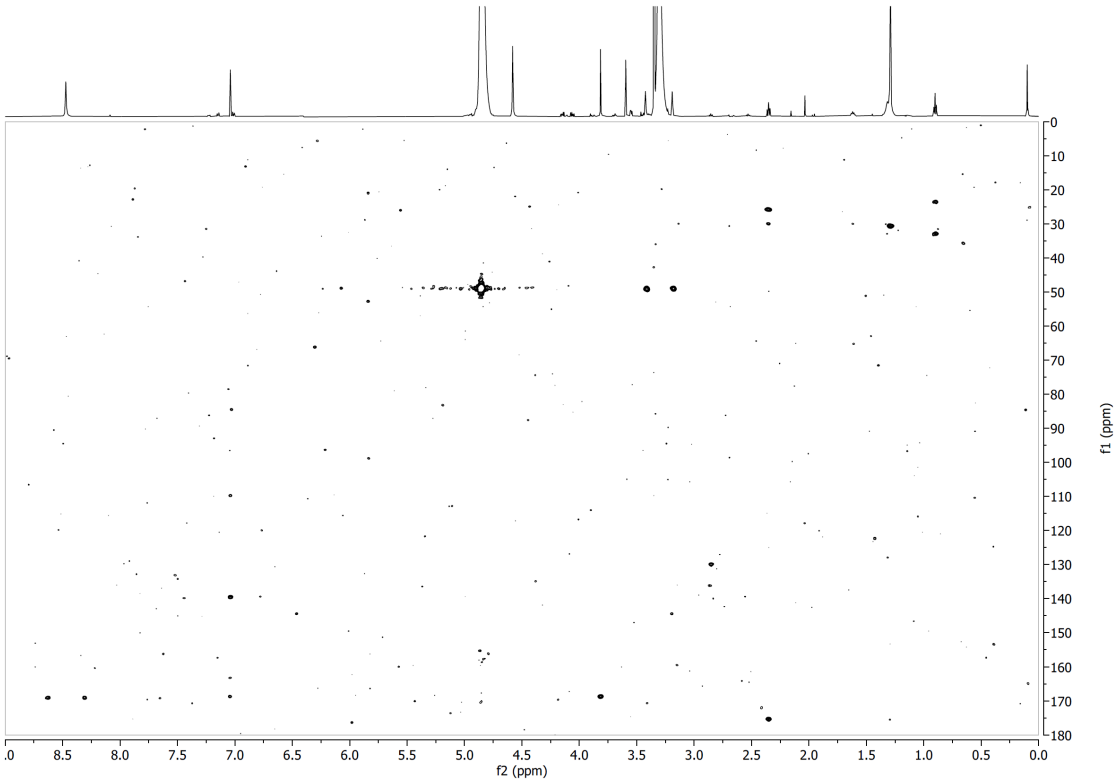
^{1}H NMR spectrum of compound **2** in CD_3OD at 600 MHz.



COSY NMR spectrum of compound **2** in CD_3OD



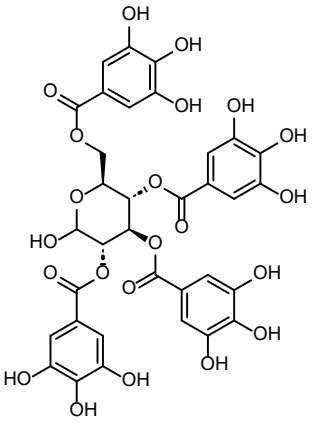
Edited-HSQC NMR spectrum of compound **2** in CD_3OD.

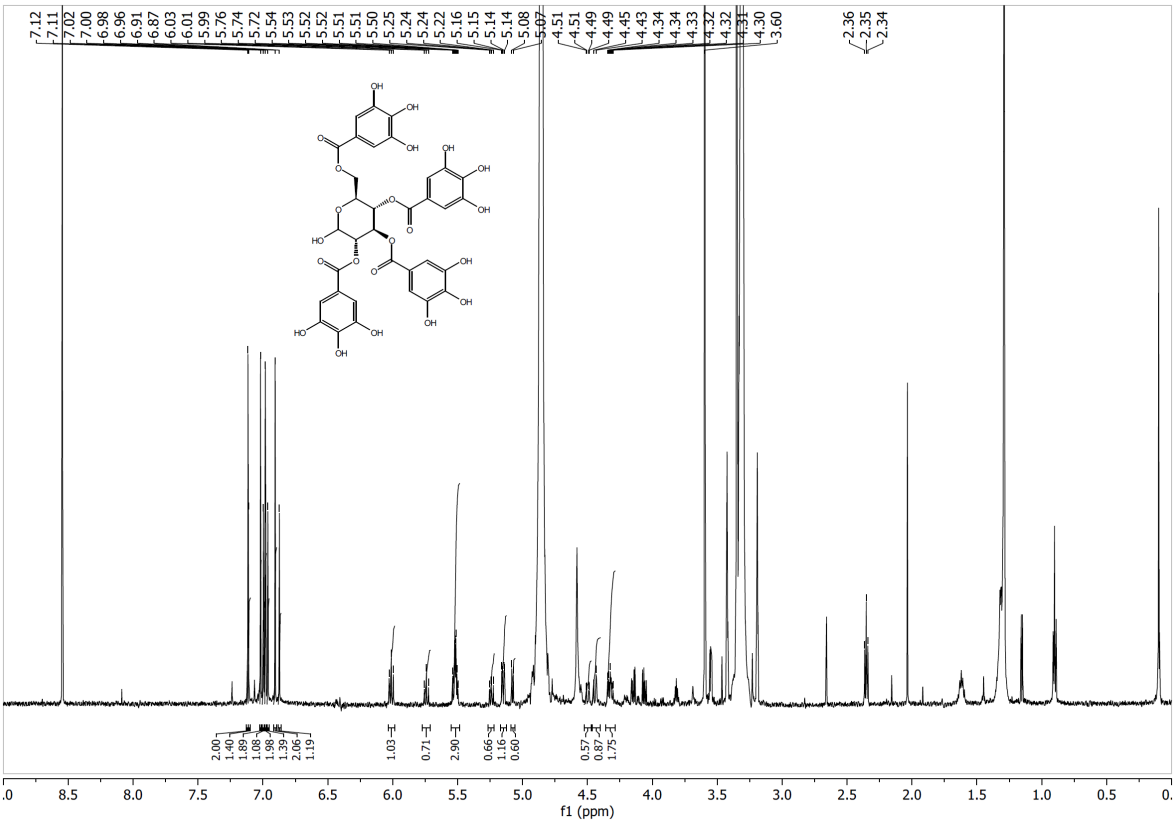


HMBC NMR spectrum of compound **2** in CD_3OD.

Figure S6: **^{1}H NMR data and spectrum of compound 3 in CD_3OD at 600 MHz.**

2,3,4,6 tetragalloyl-glucopyranoside (**3**) [292]: Mixture of α and β-glucopyranoside (1.0/0.7). ^{1}H NMR (CD_3OD, 600 MHz) δ 4.32 (2H, m, α-H-6b, β-H-6b), 4.44 (1H, d, *J* = 11.1 Hz, α-H-6a), 4.50 (1H, dd, *J* = 12.3, 2.4 Hz, β-H-6a), 5.08 (1H, d, *J* = 7.9 Hz, β-H-1), 5.15 (1H, dd, *J* = 10.0, 3.5 Hz, α-H-2), 5.24 (1H, dd, *J* = 9.8, 7.9 Hz, β-H-2), 5.52 (3H, m, α-H-1, α-H-4, β-H-4), 5.74 (1H, t, *J* = 9.8 Hz, β-H-3), 6.01 (1H, t, *J* = 10.0 Hz, α-H-3), H-2'/H-6' of galloyl from α-form: 6.91 (2H, s), 6.98 (2H, s), 7.02 (2H, s), 7.12 (2H, s), H-2'/H-6' of galloyl from β-form: 6.87 (2H, s), 6.96 (2H, s), 7.00 (2H, s), 7.11 (2H, s). HRESIMS *m/z* 787.1049 and 787.1051 $[M-H]^-$ (calcd for $C_{34}H_{27}O_{22}^-$, 787.09940, Δ = 6.3 ppm).

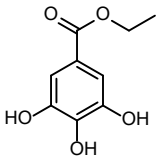


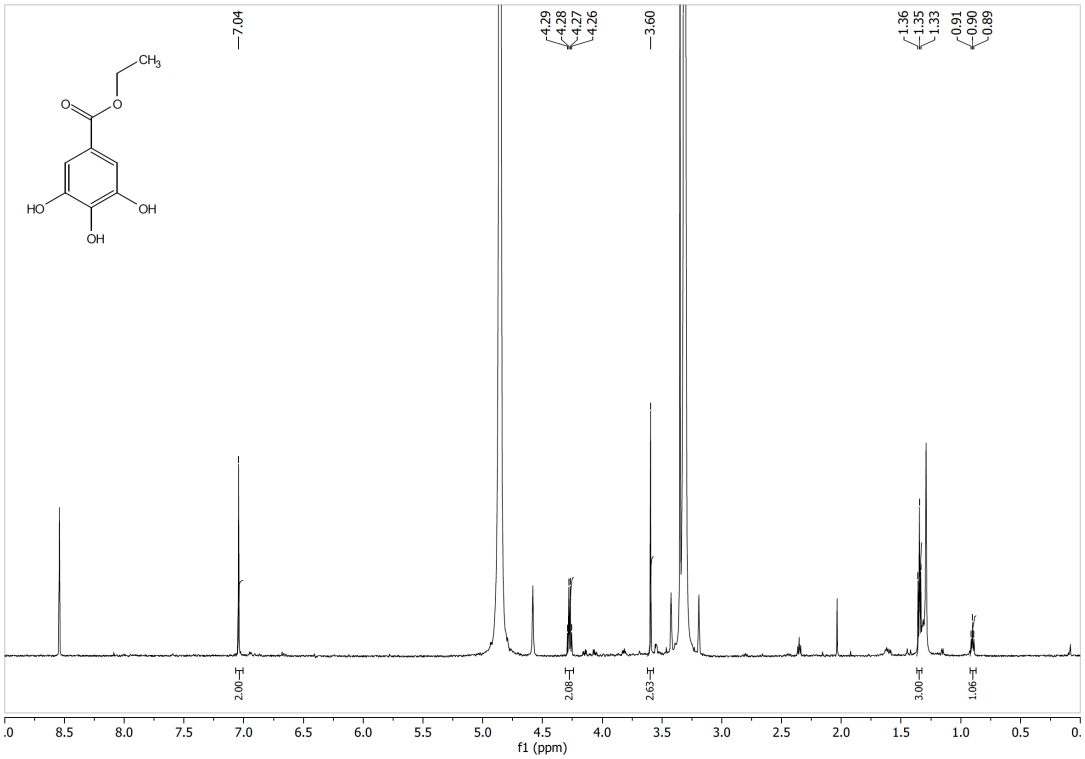


^{1}H NMR spectrum of compound **3** in CD_3OD at 600 MHz.

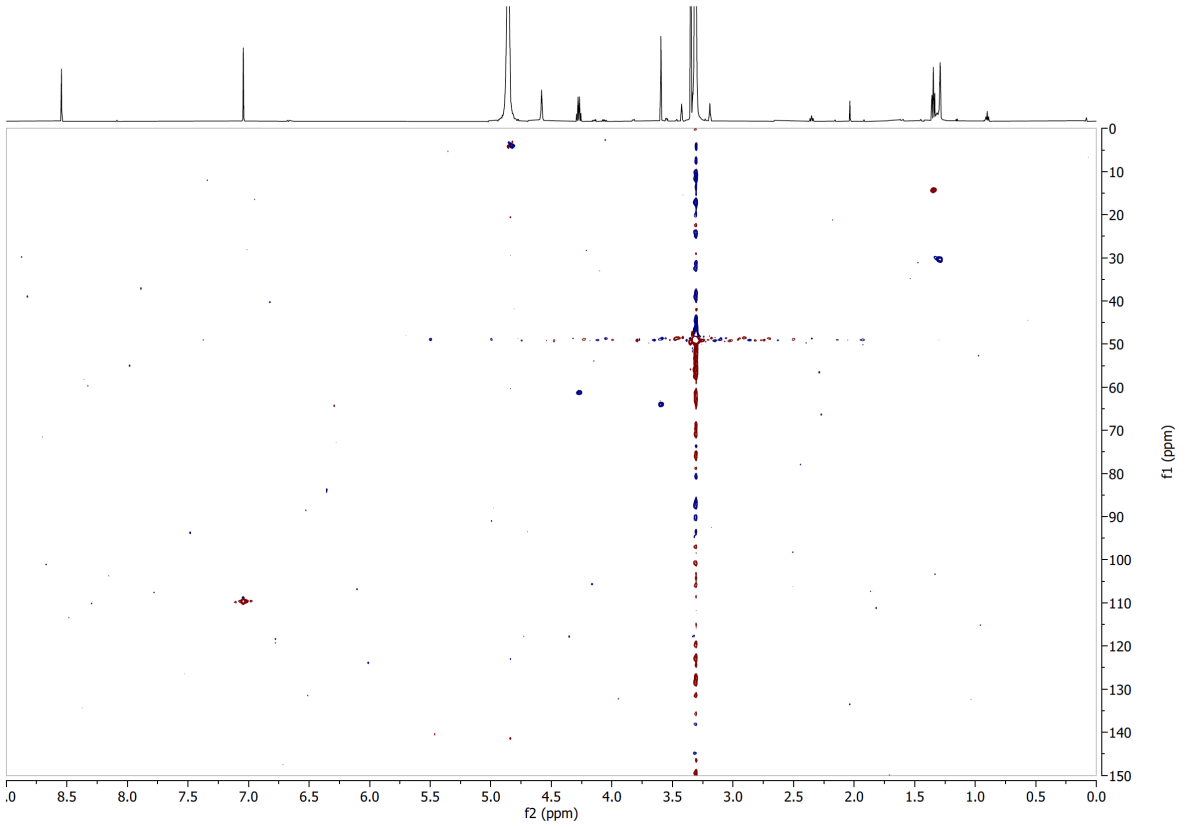
Figure S7: NMR data and spectra of compound 4 in CD_3OD at 600 MHz.

Ethyl gallate (**4**) (Leela *et al.*, 2013): ^{1}H NMR (CD_3OD, 600 MHz) δ 1.35 (3H, t, *J* = 7.1 Hz, CH_3-9), 4.27 (2H, q, *J* = 7.1 Hz, H-8), 7.04 (2H, s, H-2, H-6); ^{13}C NMR (CD_3OD, 151 MHz) δ 14.2 (CH_3-9), 61.2 (C-8), 109.7 (C-2, C-6), 121.5 (C-1), 139.6 (C-4), 168.2 (C-7). HRESIMS *m/z* 197.0459 $[M-H]^-$ (calcd for $C_9H_9O_5^-$, 197.04500, Δ = 1.9 ppm).

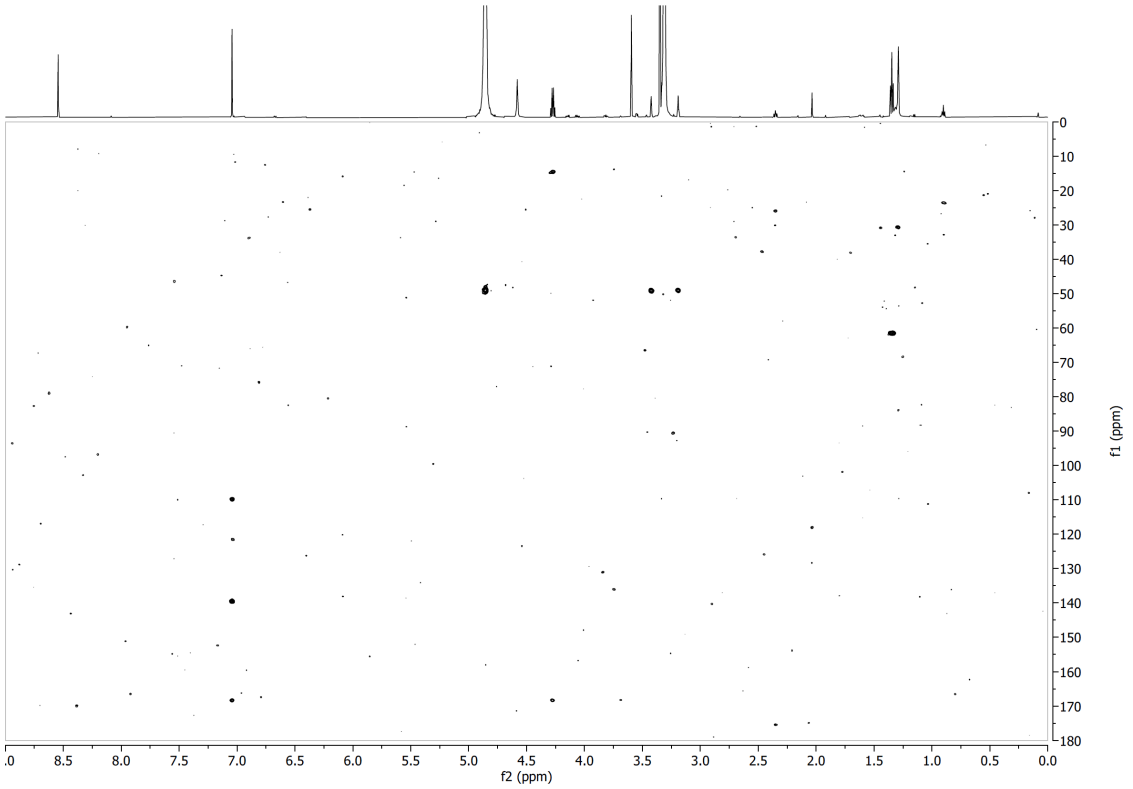




^{1}H NMR spectrum of compound **4** in CD_3OD at 600 MHz.



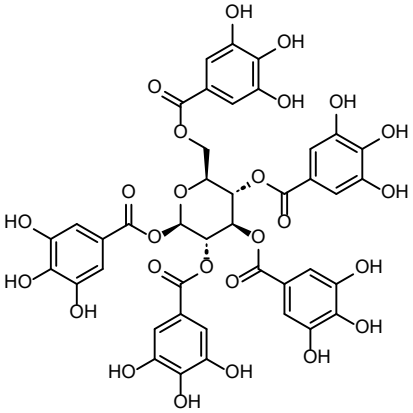
Edited-HSQC NMR spectrum of compound **4** in CD_3OD.

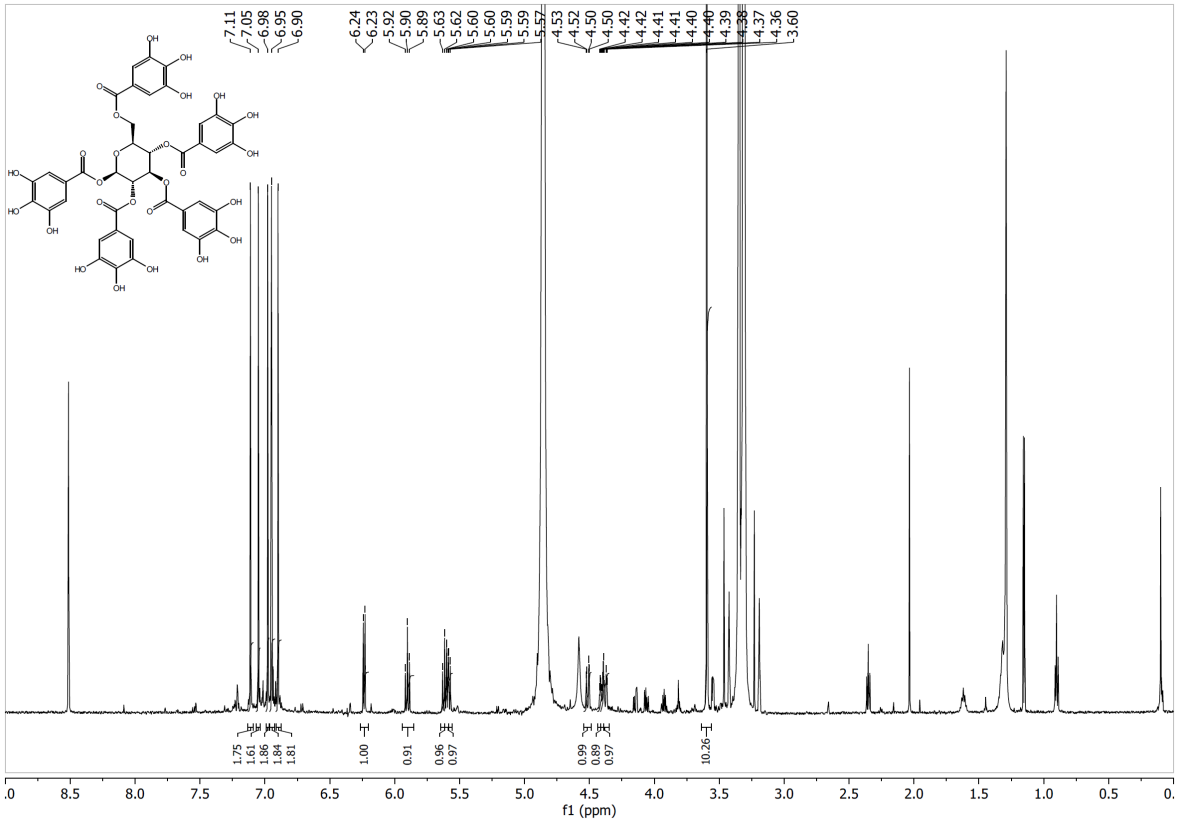


HMBC NMR spectrum of compound **4** in CD_3OD.

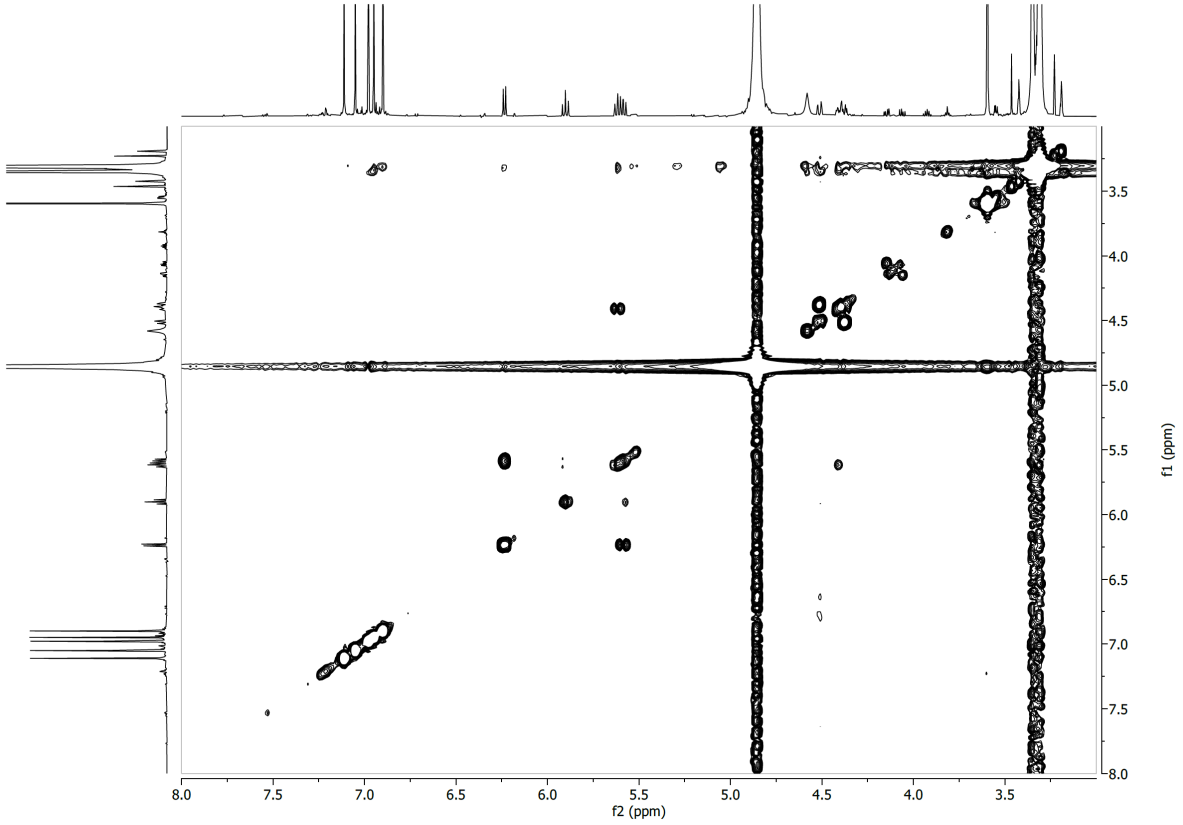
Figure S8: **NMR data and spectra of compound 5 in CD_3OD at 600 MHz.**

1,2,3,4,6 pentagalloyl-β-glucopyranoside (**5**) [293]: ^1H NMR (CD_3OD, 600 MHz) δ 4.38 (1H, dd, *J* = 12.1, 4.4 Hz, H-6b), 4.41 (1H, m, H-5), 4.51 (1H, dd, *J* = 12.1, 1.6 Hz, H-6a), 5.59 (1H, dd, *J* = 9.7, 8.3 Hz, H-2), 5.62 (1H, t, *J* = 9.7 Hz, H-4), 5.90 (1H, t, *J* = 9.7 Hz, H-3), 6.24 (1H, d, *J* = 8.3 Hz, H-1), 6.90 (2H, s, H-2'-3, H-6'-3), 6.95 (2H, s, H-2'-2, H-6'-2), 6.98 (2H, s, H-2'-4, H-6'-4), 7.05 (2H, s, H-2'-1, H-6'-1), 7.11 (2H, s, H-2'-6, H-6'-6); ^{13}C NMR (CD_3OD, 151 MHz) δ 63.1 (C-6), 69.8 (C-4), 72.2 (C-2), 74.1 (C-3), 74.4 (C-5), 93.8 (C-1), 110.3 (C-2'-6, C-6'-6), 110.4 (C-2'-3, C-6'-3), 110.4 (C-2'-2, C-6'-2), 110.5 (C-2'-4, C-6'-4), 110.6 (C-2'-1, C-6'-1), 140.0 (C-4'-6), 140.1 (C-4'-3), 140.3 (C-4'-4), 140.4 (C-4'-2), 140.8 (C-4'-1), 146.3 (C-3'-3, C-5'-3), 146.4 (C-3'-2, C-5'-2), 146.5 (C-3'-4, C-5'-4), 146.5 (C-3'-6, C-5'-6), 146.6 (C-3'-1, C-5'-1), 166.2 (C-7'-1), 166.9 (C-7'-4), 167.0 (C-7'-2), 167.3(C-7'-3), 167.9 (C-7'-6). HRESIMS *m/z* 939.1157 $[M-H]^-$ (calcd for $C_{41}H_{31}O_{26}^-$, 939.11036, Δ = 5.1 ppm), only observed as *m/z* 469.0521 $[M-H]^{2-}$ in the decoction extract of *B. schreberi*.

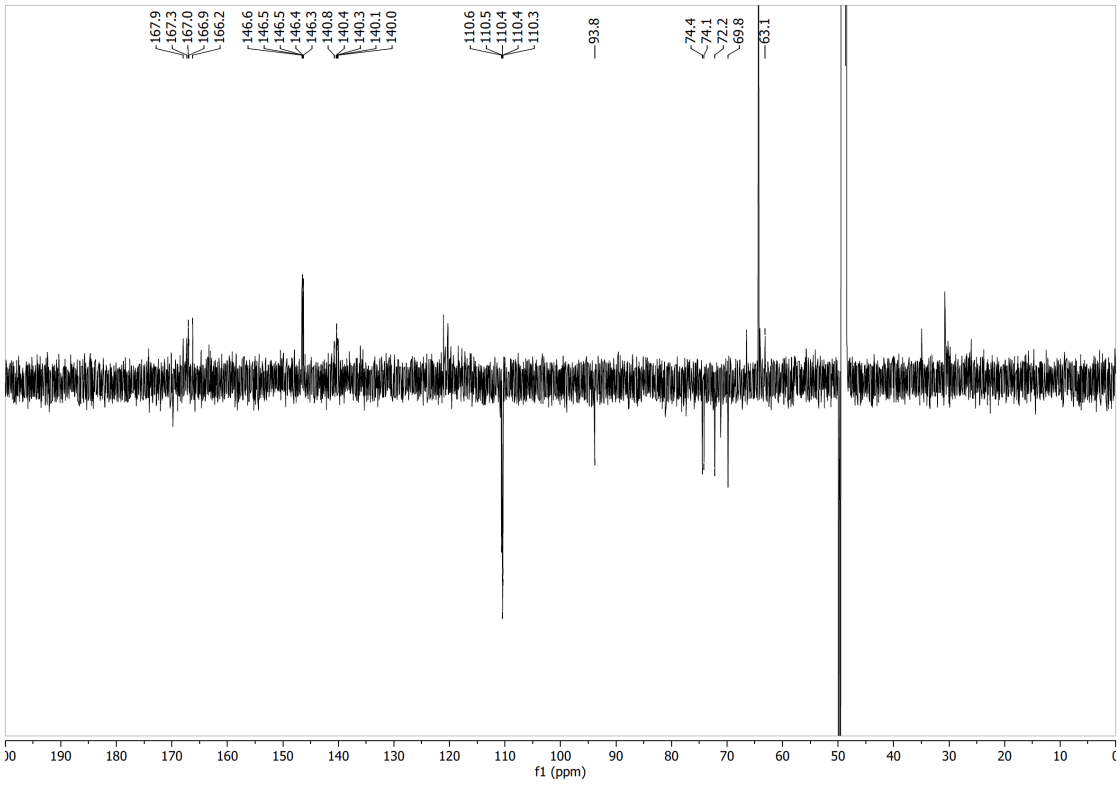




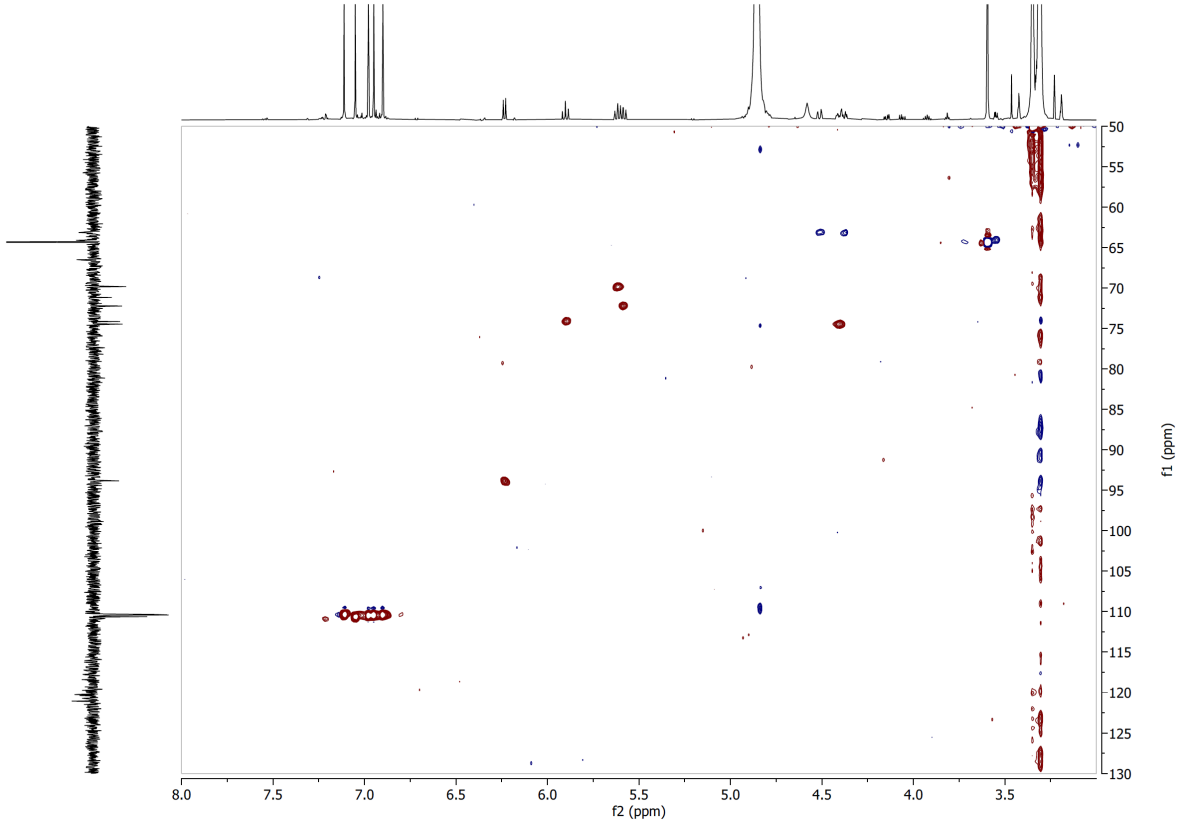
^1H NMR spectrum of compound **5** in CD_3OD at 600 MHz.



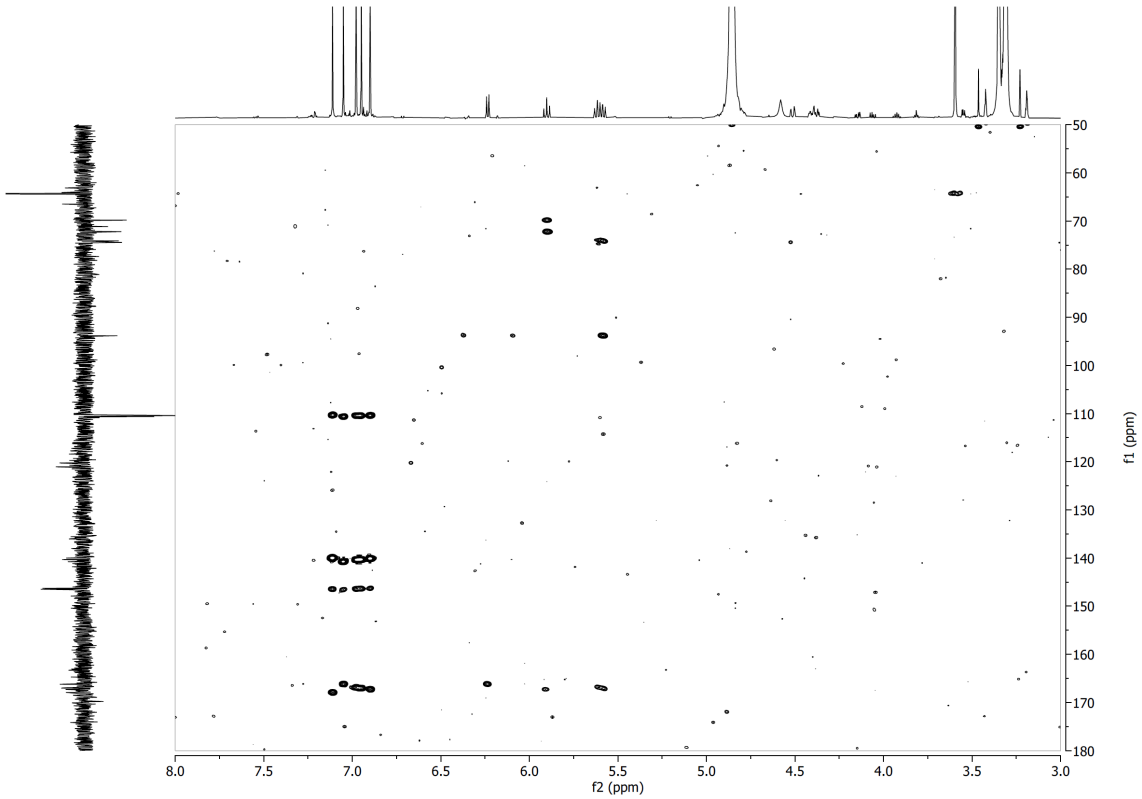
COSY NMR spectrum of compound **5** in CD_3OD.



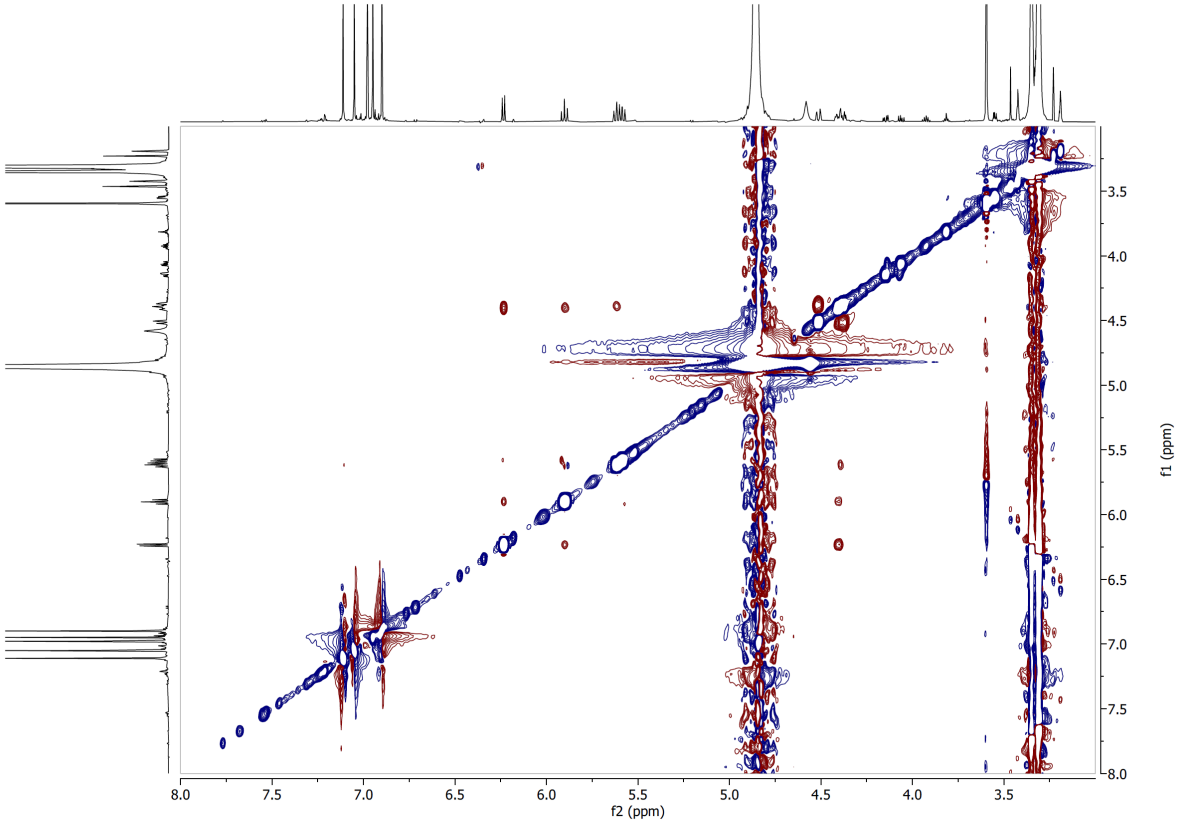
^{13}C-DEPTQ NMR spectrum of compound **5** in CD_3OD at 151 MHz.



Edited-HSQC NMR spectrum of compound **5** in CD_3OD.



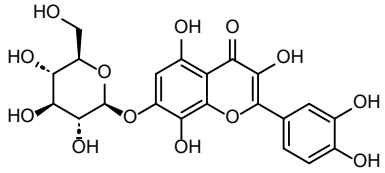
HMBC NMR spectrum of compound **5** in CD_3OD.

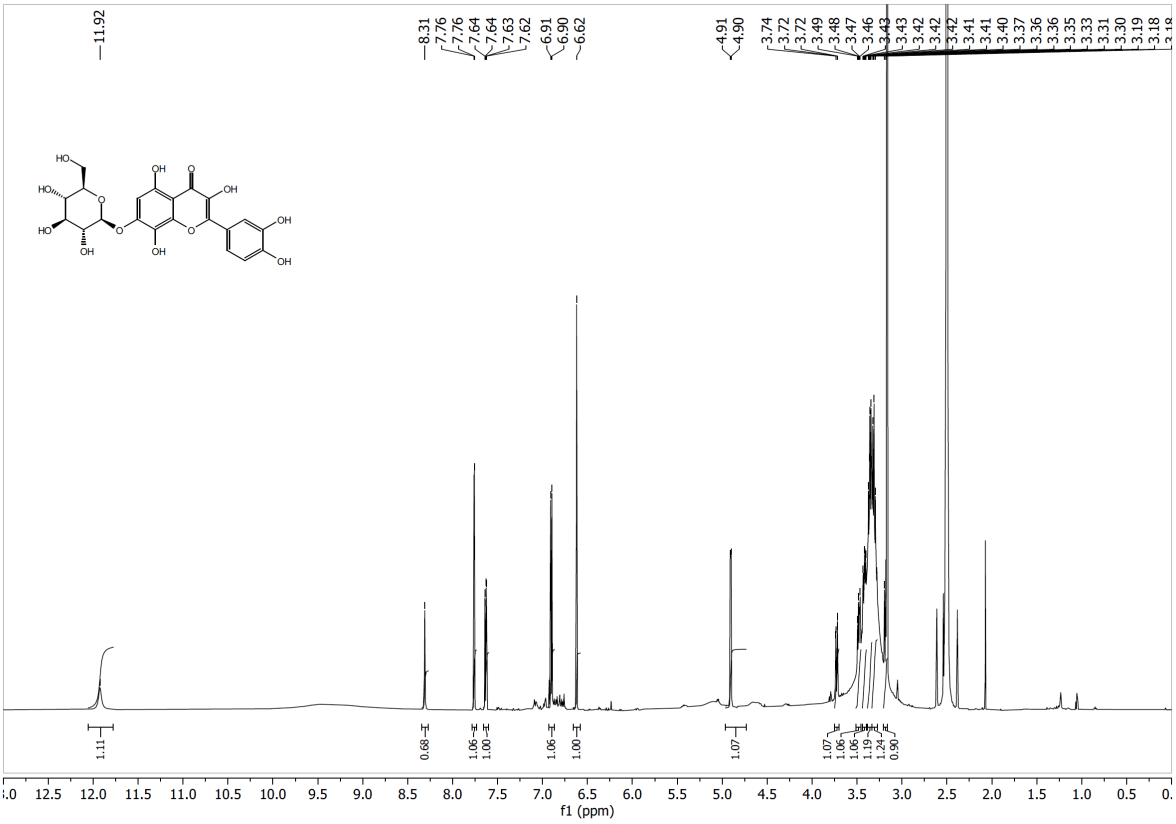


ROESY NMR spectrum of compound **5** in CD_3OD.

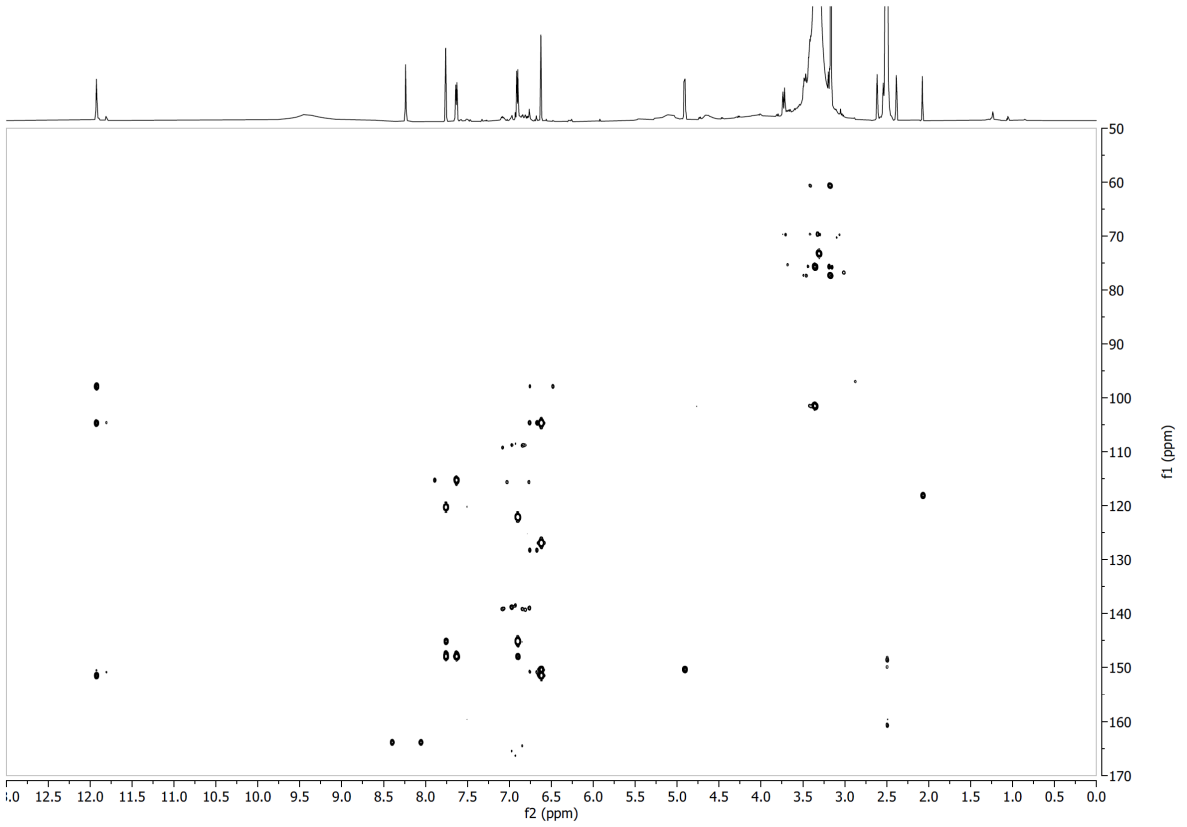
Figure S9: **NMR data and spectra of compound 6 in DMSO-d_6 at 600 MHz.**

Gossypetin-7-*O*-β-glucopyranoside (**6**) [294]: ^{1}H NMR (DMSO-d_6, 600 MHz) δ 3.18 (1H, t, *J* = 9.8, 8.8 Hz, H-4''), 3.31 (1H, t, *J* = 9.2, 8.8 Hz, H-3''), 3.36 (1H, dd, *J* = 9.2, 7.6 Hz, H-2''), 3.42 (1H, ddd, *J* = 9.8, 6.0, 2.1 Hz, H-5''), 3.48 (1H, dd, *J* = 11.8, 6.0 Hz, H-6''b), 3.73 (1H, dd, *J* = 11.8, 2.1 Hz, H-6''a), 4.91 (1H, d, *J* = 7.6 Hz, H-1''), 6.62 (1H, s, H-6), 6.90 (1H, d, *J* = 8.5 Hz, H-5'), 7.63 (1H, dd, *J* = 8.5, 2.2 Hz, H-6'), 7.76 (1H, d, *J* = 2.2 Hz, H-2'), 11.92 (1H, s, 5OH); ^{13}C NMR (DMSO-d_6, 151 MHz) δ 60.7 (C-6''), 69.7 (C-4''), 73.3 (C-2''), 75.7 (C-3''), 77.3 (C-5''), 97.8 (C-6), 101.4 (C-1''), 104.7 (C-10), 115.3 (C-2'), 120.3 (C-6'), 122.1 (C-1'), 126.8 (C-8), 145.0 (C-3'), 147.9 (C-4'), 150.4 (C-7), 151.5 (C-5). HRESIMS *m/z* 479.0845 [M-H]$^-$ (calcd for $C_{21}H_{19}O_{13}^-$, 479.08257, Δ = 2.9 ppm).

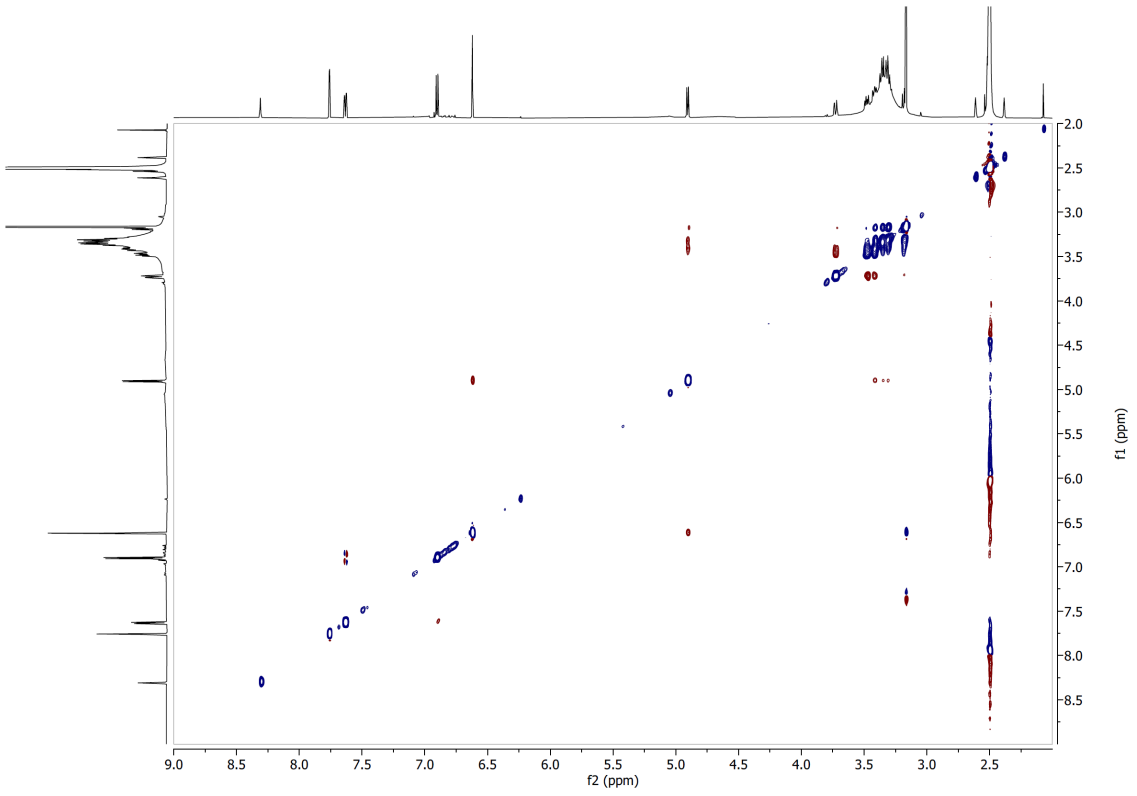




^{1}H NMR spectrum of compound **6** in DMSO-d_6 at 600 MHz.



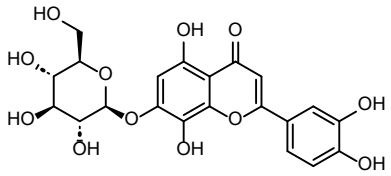
HMBC NMR spectrum of compound **6** in DMSO-d_6.

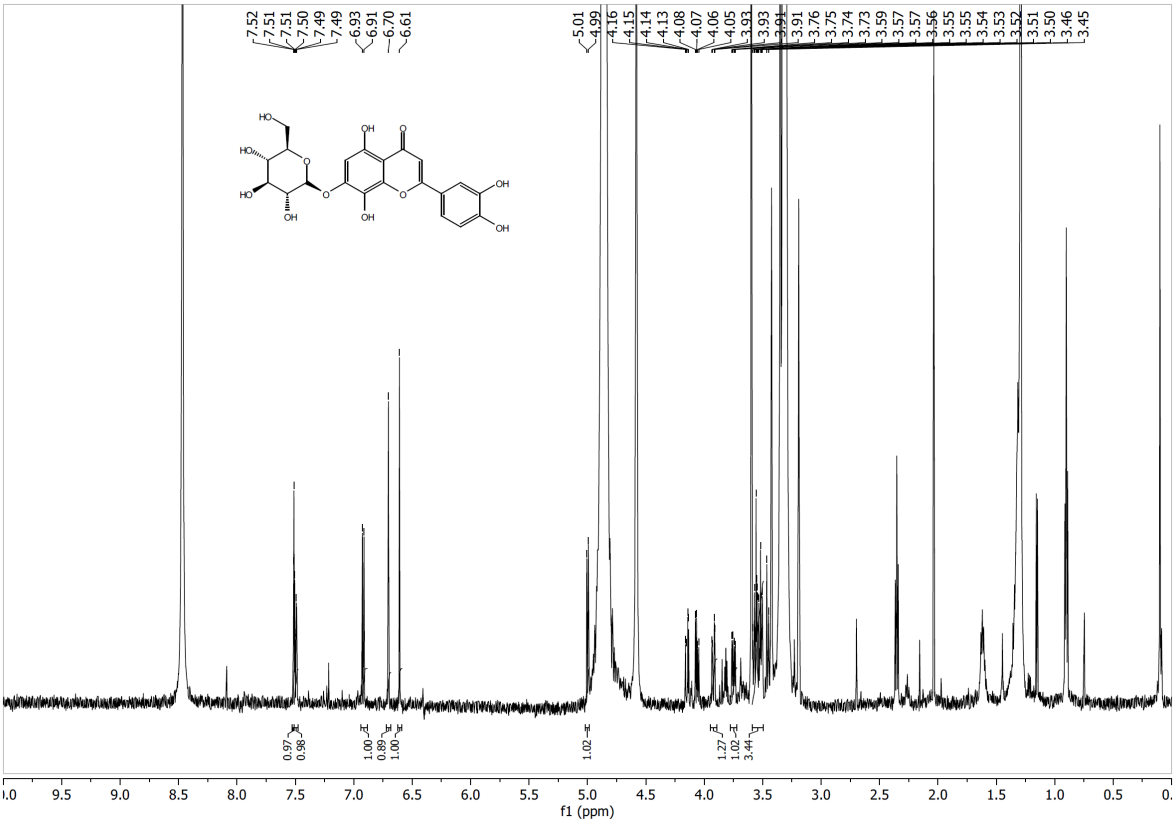


ROESY NMR spectrum of compound **6** in DMSO-d_6.

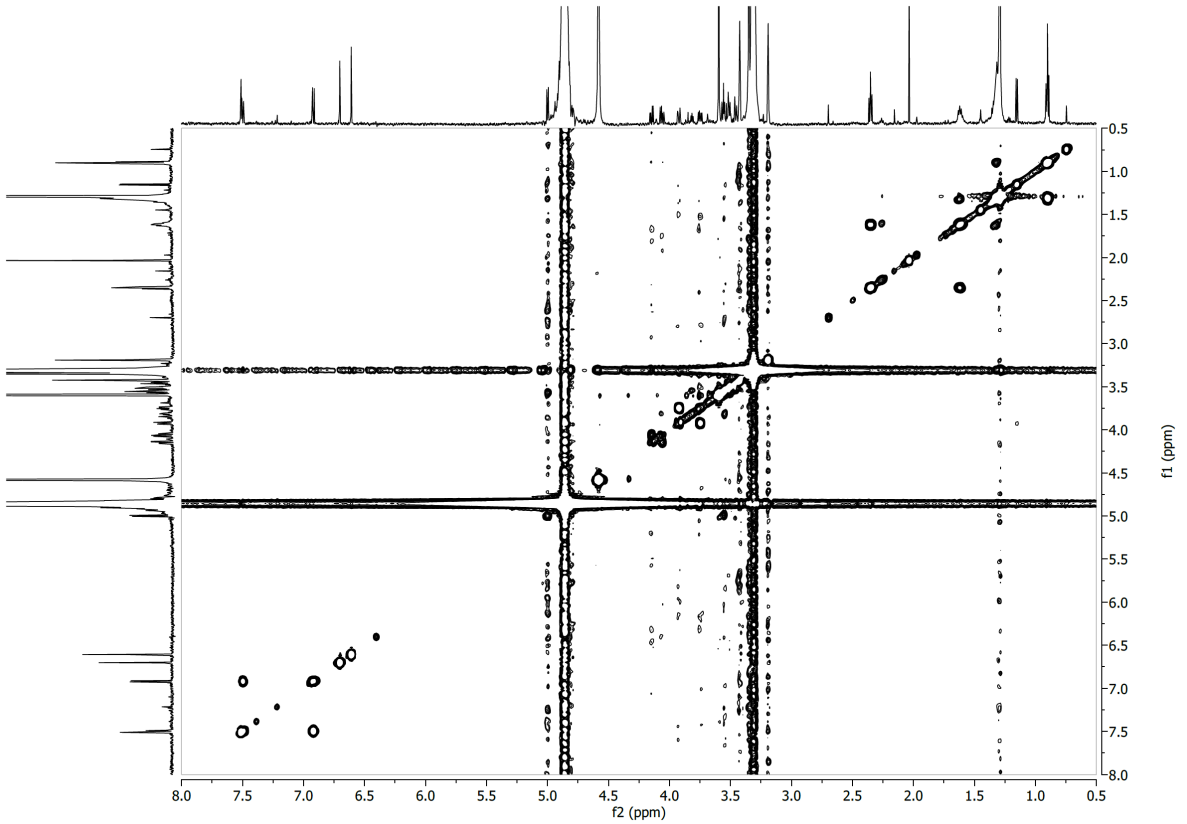
Figure S10: **NMR data and spectra of compound 7 in CD_3OD at 600 MHz**.

Hypolaetin-7-*O*-glucoside (**7**) (Zapesochnaya et al., 1973): ^{1}H NMR (CD_3OD, 600 MHz) δ 3.55 (4H, m, H-2'', H-3'', H-4'', H-5''), 3.75 (1H, dd, *J* = 12.2, 5.3 Hz, H-6''b), 3.92 (1H, dd, *J* = 12.2, 2.3 Hz, H-6''a), 5.00 (1H, d, *J* = 7.6 Hz, H-1''), 6.61 (1H, s, H-3), 6.70 (1H, s, H-6), 6.92 (1H, d, *J* = 8.2 Hz, H-5'), 7.50 (1H, dd, *J* = 8.2, 2.3 Hz, H-6'), 7.51 (1H, d, *J* = 2.3 Hz, H-2'); ^{13}C NMR (CD_3OD, 151 MHz) δ 61.9 (C-6''), 71.0 (C-4''), 74.4 (C-2''), 77.2 (C-3''), 78.2 (C-5''), 100.0 (C-6), 102.7 (C-1''), 103.4 (C-3), 107.0 (C-10), 114.1 (C-2'), 116.4 (C-5'), 120.3 (C-6'), 123.4 (C-1'), 128.6 (C-8), 146.8 (C-3'), 150.8 (C-4'), 166.6 (C-2). HRESIMS *m/z* 463.0897 [M-H]$^-$ (calcd for $C_{21}H_{19}O_{12}^-$, 463.08765, Δ = 3.3 ppm).

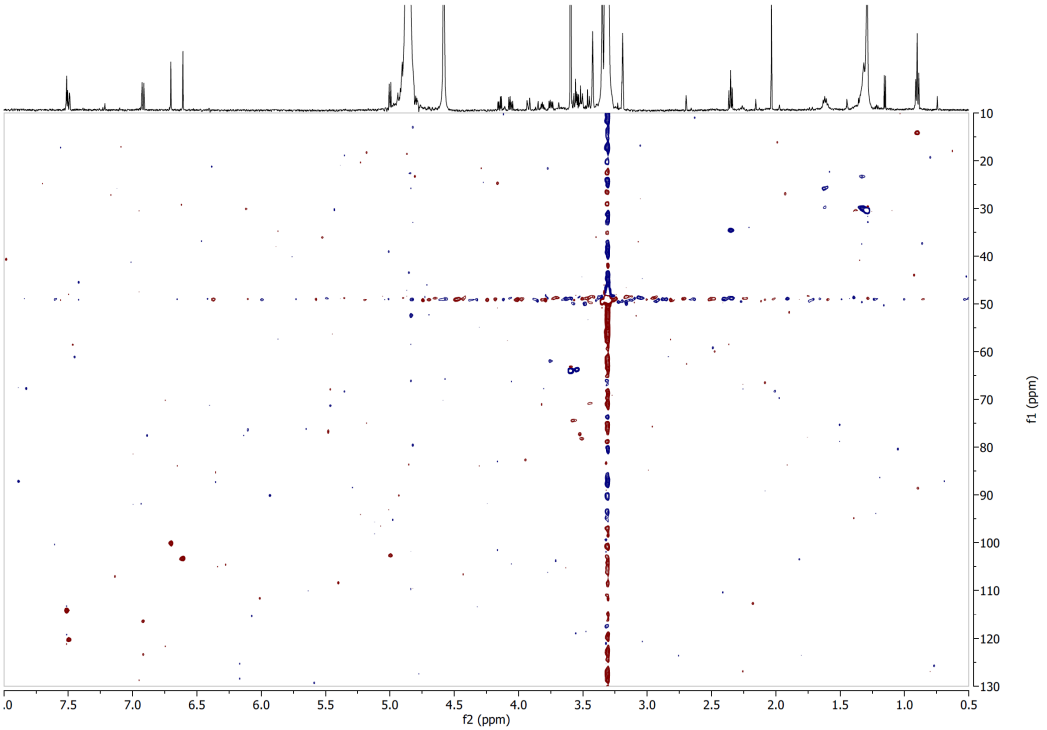




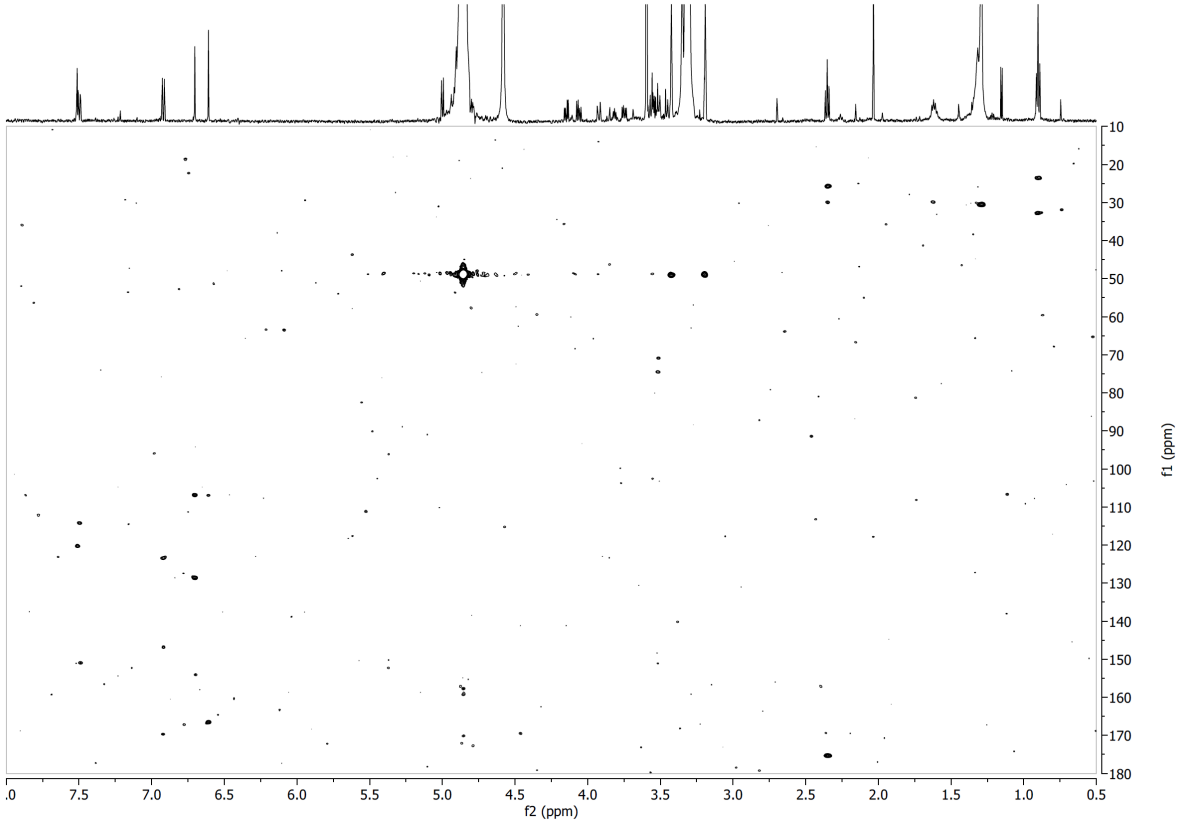
^{1}H NMR spectrum of compound **7** in CD_3OD at 600 MHz.



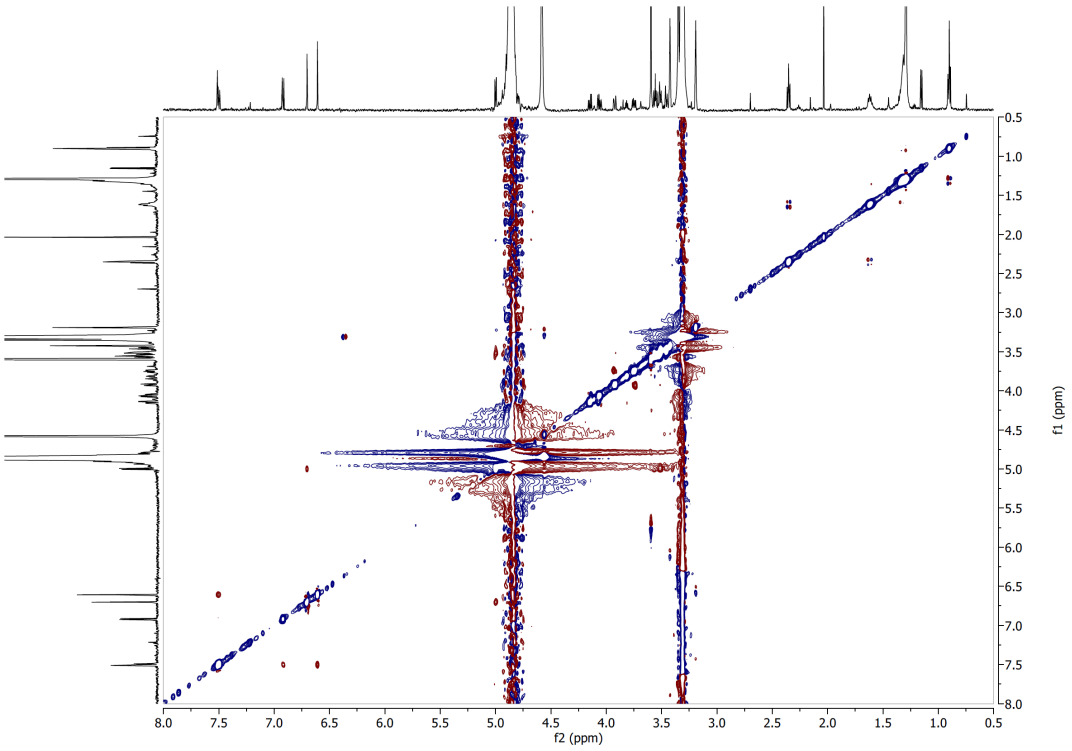
COSY NMR spectrum of compound **7** in CD_3OD.



Edited-HSQC NMR spectrum of compound **7** in CD_3OD.



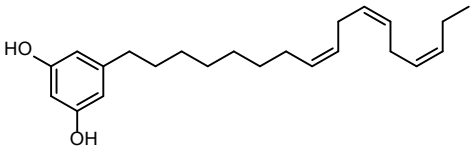
HMBC NMR spectrum of compound **7** in CD_3OD.

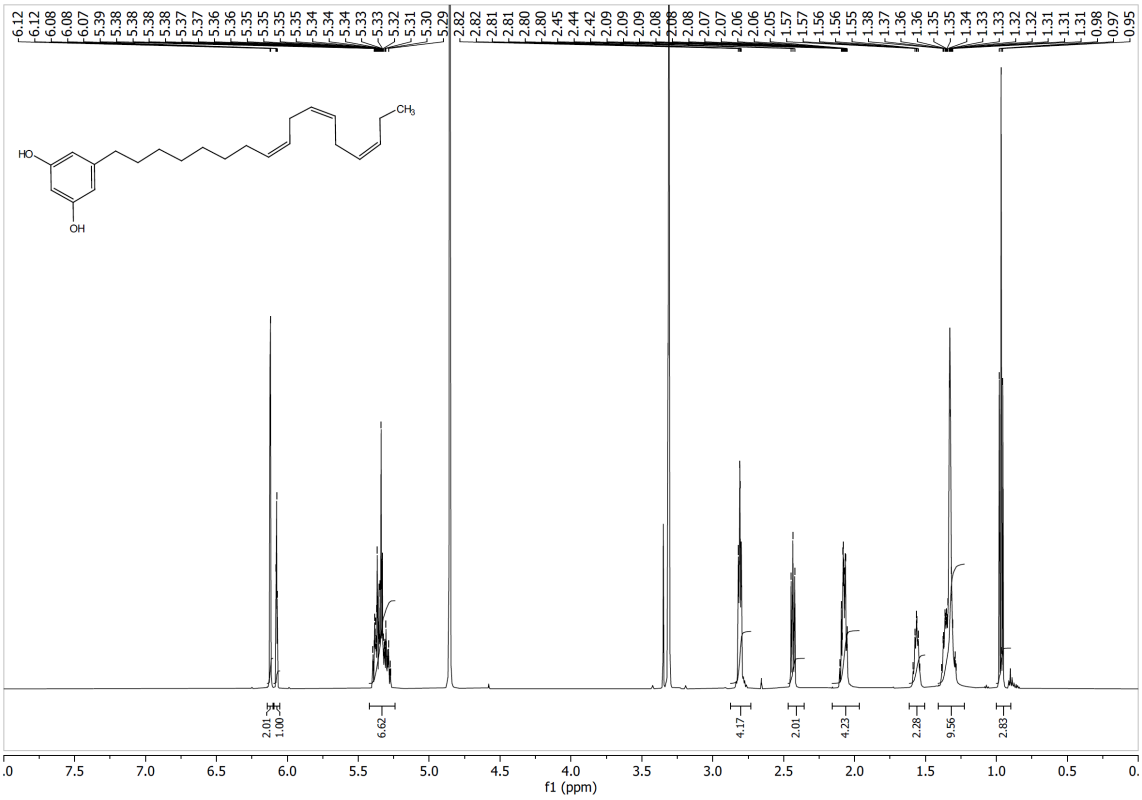


ROESY NMR spectrum of compound **7** in CD_3OD.

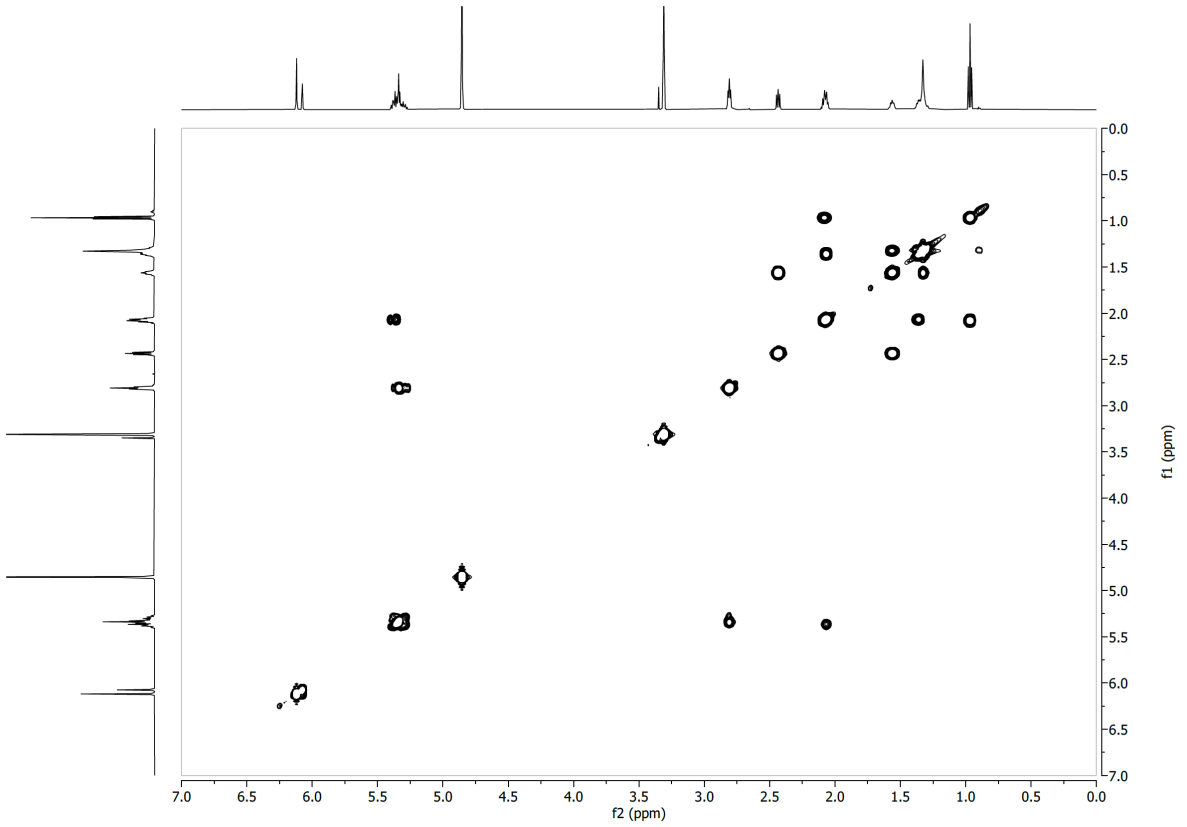
Figure S11: NMR data and spectra of compound 8 in CD_3OD at 600 MHz.

5-[(8Z,11Z,14Z)-heptadeca-8,11,14-trienyl] resorcinol (**8**) [295]: ^1H NMR (CD_3OD, 600 MHz) δ 0.97 (3H, t, *J* = 7.5 Hz, CH_3-25), 1.34 (8H, m, H-9, H-10, H-11, H-12), 1.57 (2H, m, H-8), 2.08 (4H, m, H-13, H-24), 2.44 (2H, m, H-7), 2.81 (4H, m, H-16, H-19), 5.35 (6H, m, H-14, H-15, H-17, H-18, H-22, H-23), 6.08 (1H, t, *J* = 2.2 Hz, H-4), 6.12 (2H, d, *J* = 2.2 Hz, H-2, H-6); ^{13}C NMR (CD_3OD, 151 MHz) δ 14.6 (CH_3-25), 21.5 (C-24), 26.4 (C-16), 26.5 (C-19), 28.2 (C-13), 30.3 (C-9), 30.3 (C-11), 30.5 (C-10), 30.7 (C-12), 32.4 (C-8), 37.0 (C-7), 100.9 (C-4), 107.9 (C-2, C-6), 128.2 (C-22), 128.8 (C-15), 129.2 (C-18), 129.2 (C-17), 131.1 (C-14), 132.7 (C-23), 146.3 (C-1), 159.3 (C-3, C-5). HRESIMS *m/z* 341.24808 $[M-H]^-$ (calcd for $C_{23}H_{33}O_2^-$, 341.24806, Δ = 1.7 ppm).

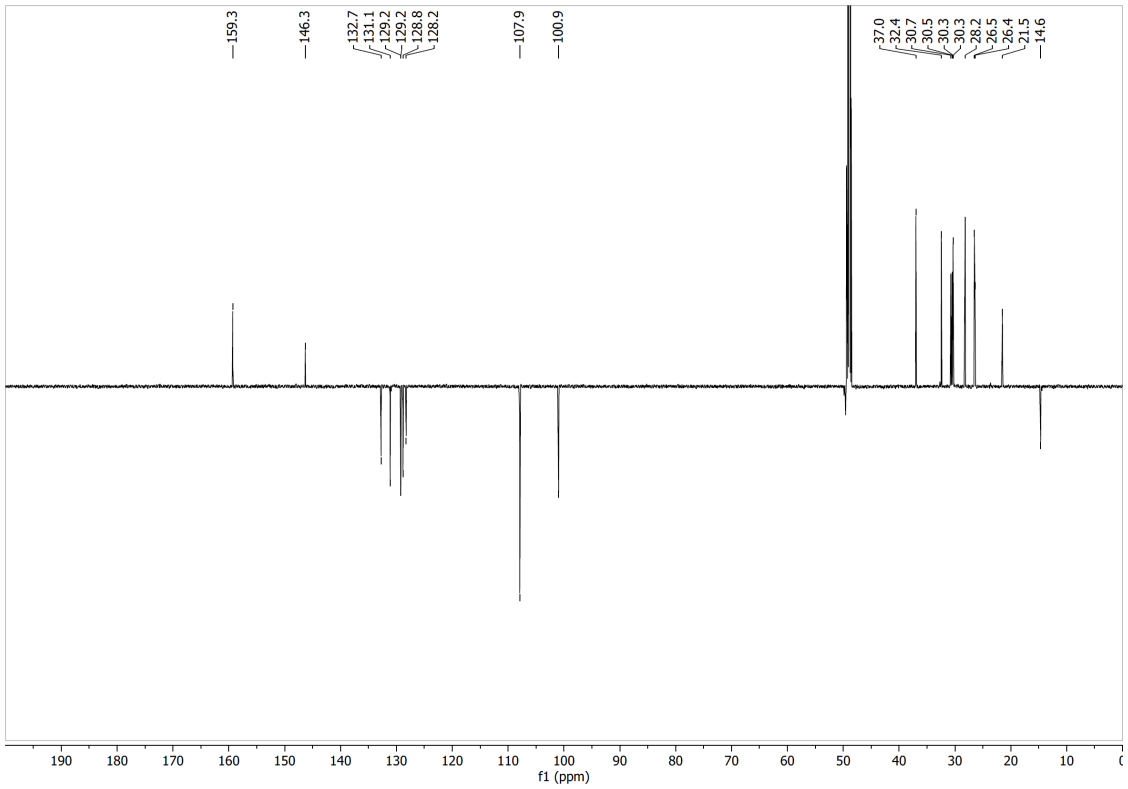




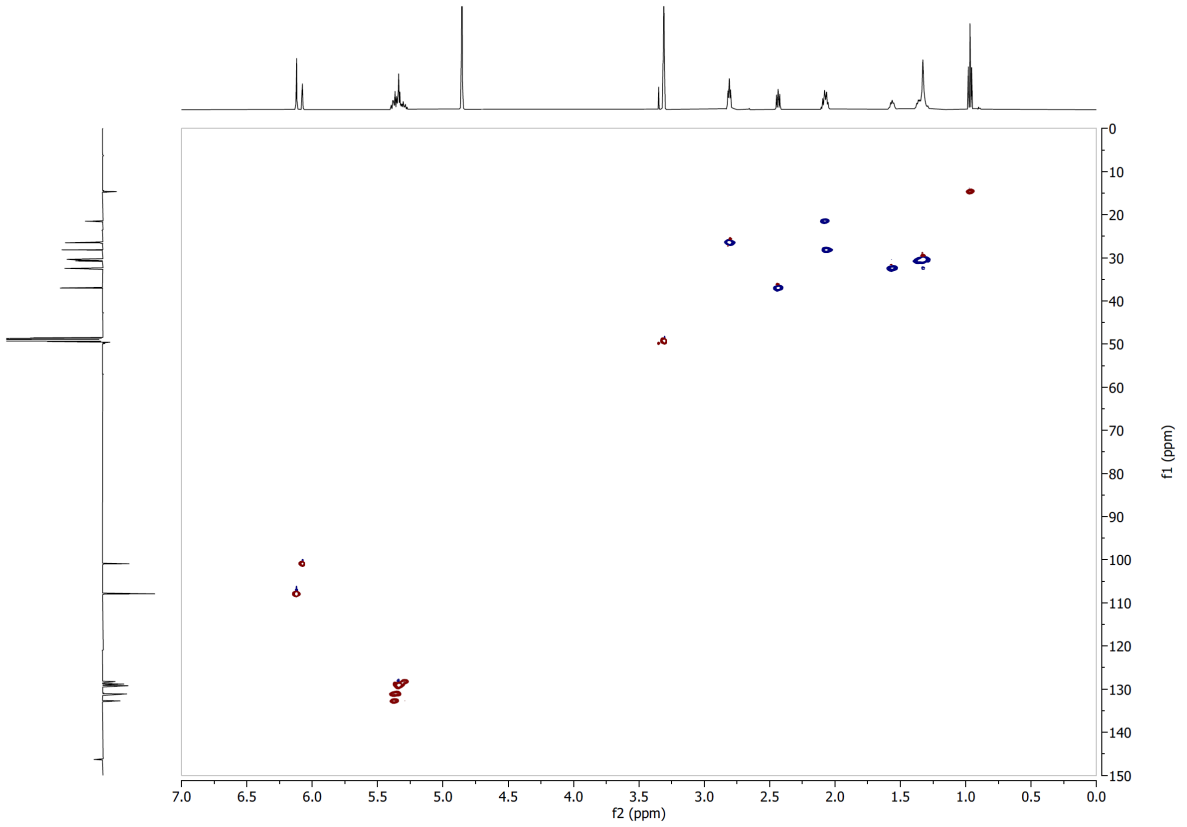
^1H NMR spectrum of compound **8** in CD_3OD at 600 MHz.



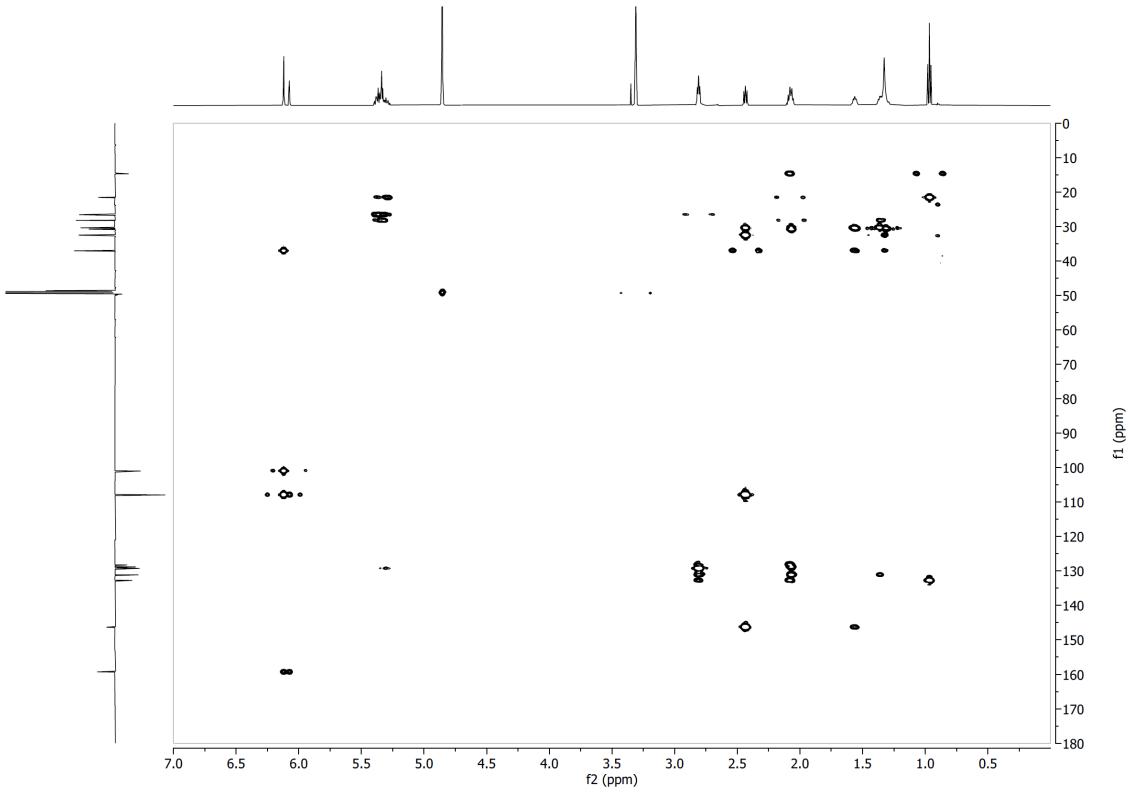
COSY NMR spectrum of compound **8** in CD_3OD.



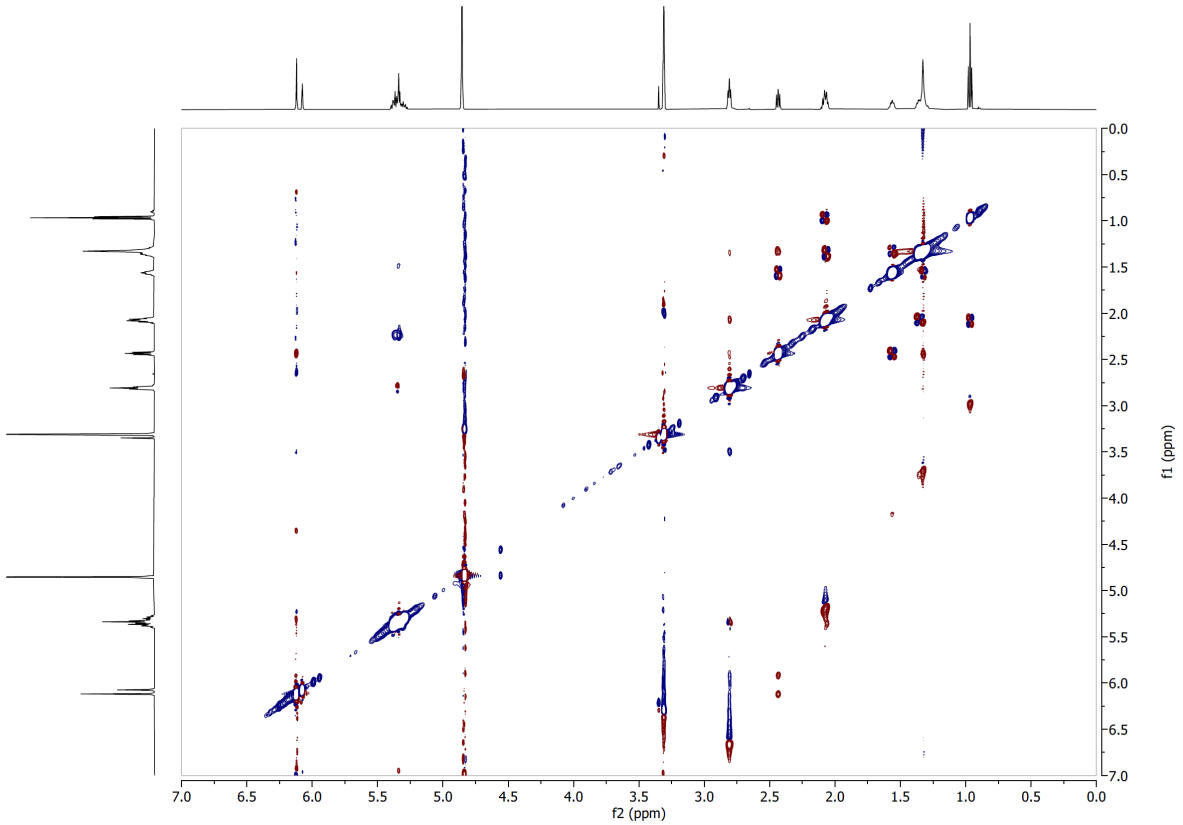
^{13}C-DEPTQ NMR spectrum of compound **8** in CD_3OD at 151 MHz.



Edited-HSQC NMR spectrum of compound **8** in CD_3OD.

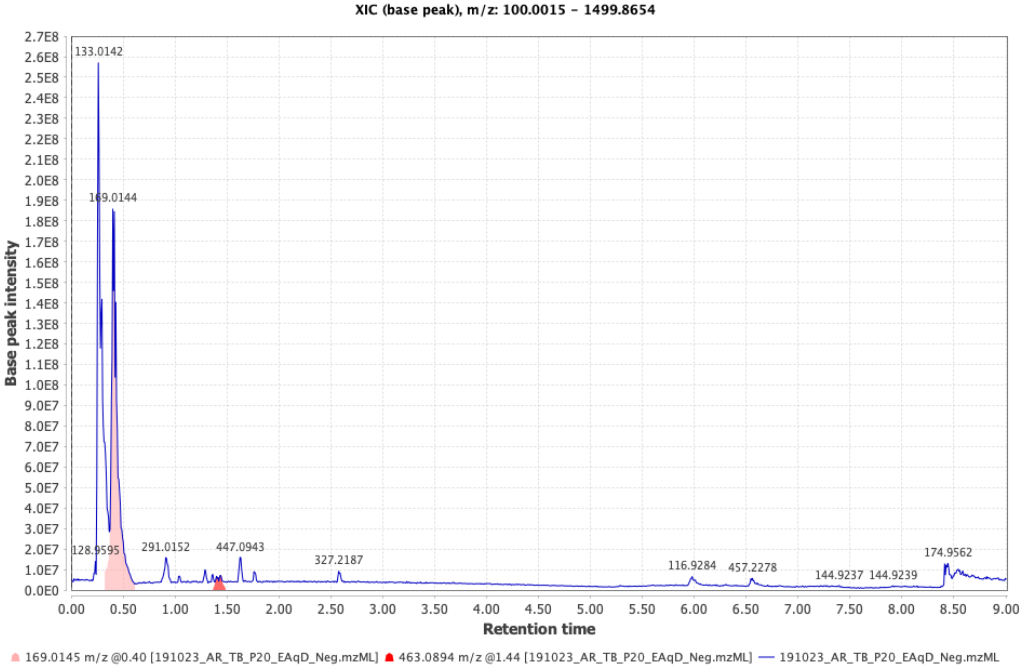


HMBC NMR spectrum of compound **8** in CD_3OD.

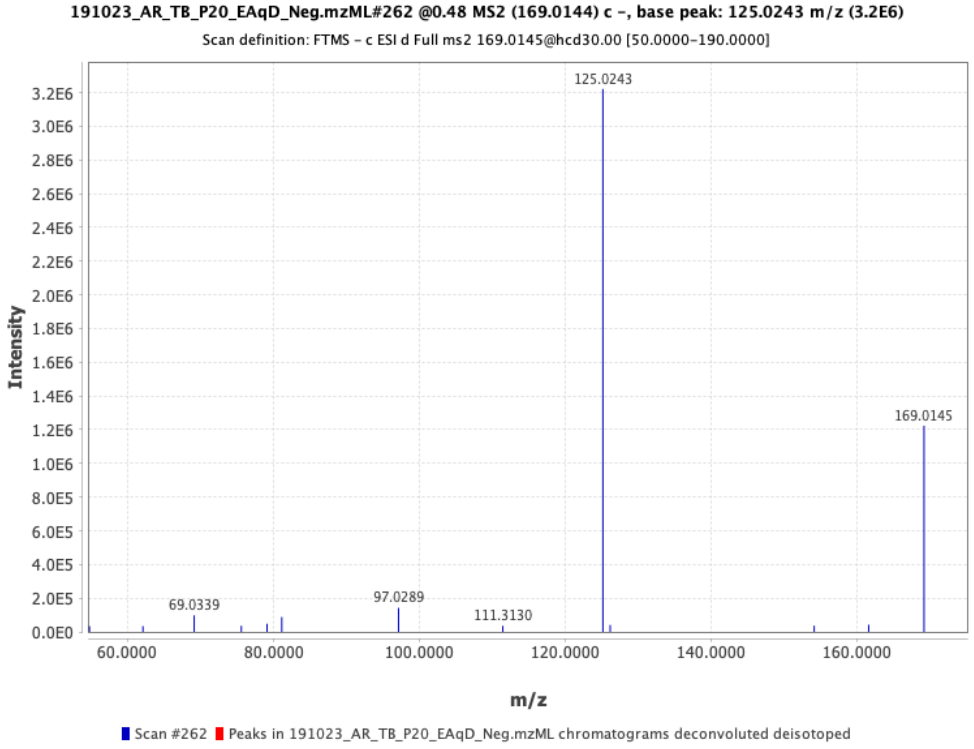


ROESY NMR spectrum of compound **8** in CD_3OD.

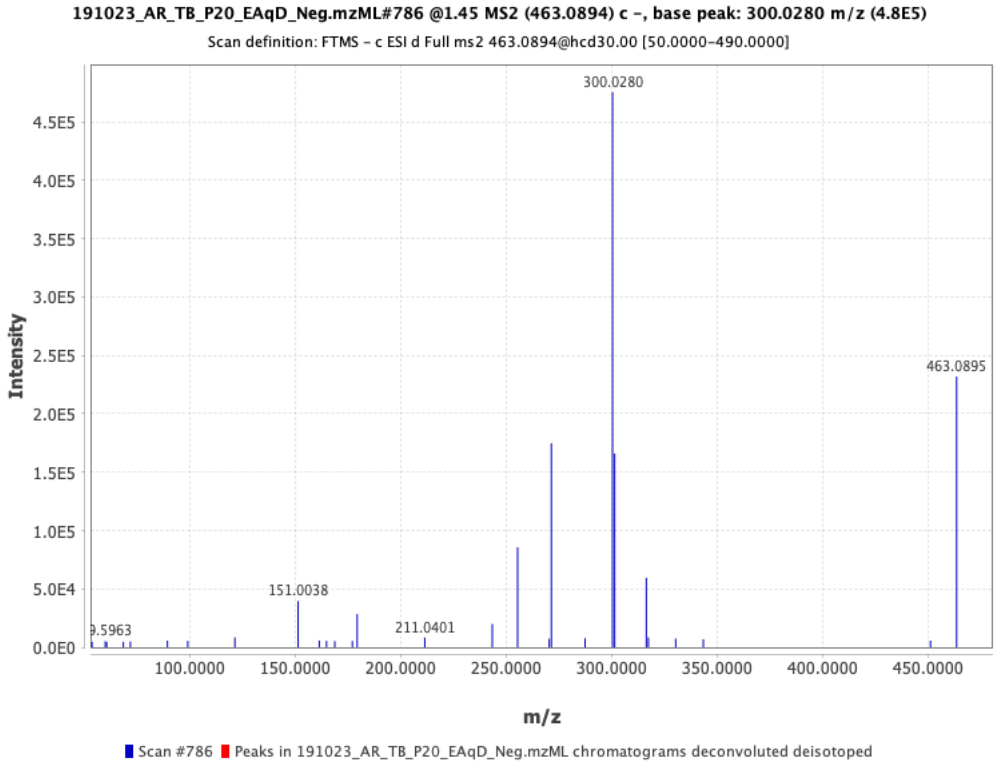
Figure S12: **UHPLC-HRMS chromatogram showing the presence of compounds 1 and 7 in the decoction of *N. lotus* and correspondence of their MS/MS spectra.**



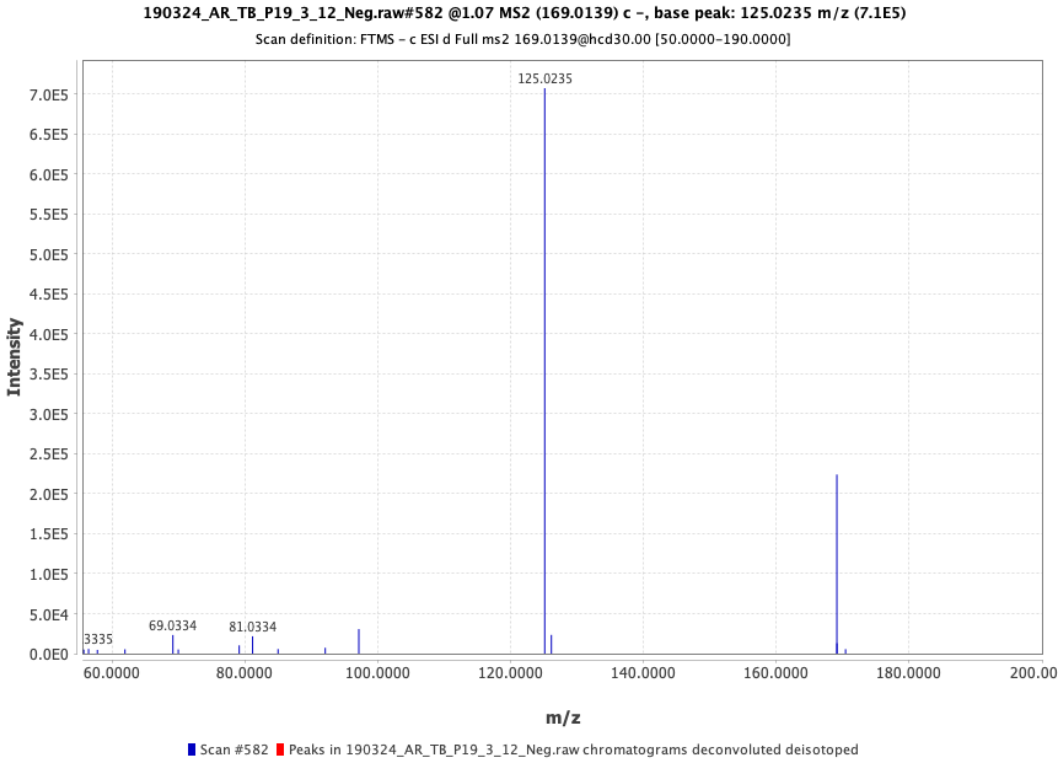
Chromatogram of the decoction of *N. lotus* highlighting compounds **1** and **7**.



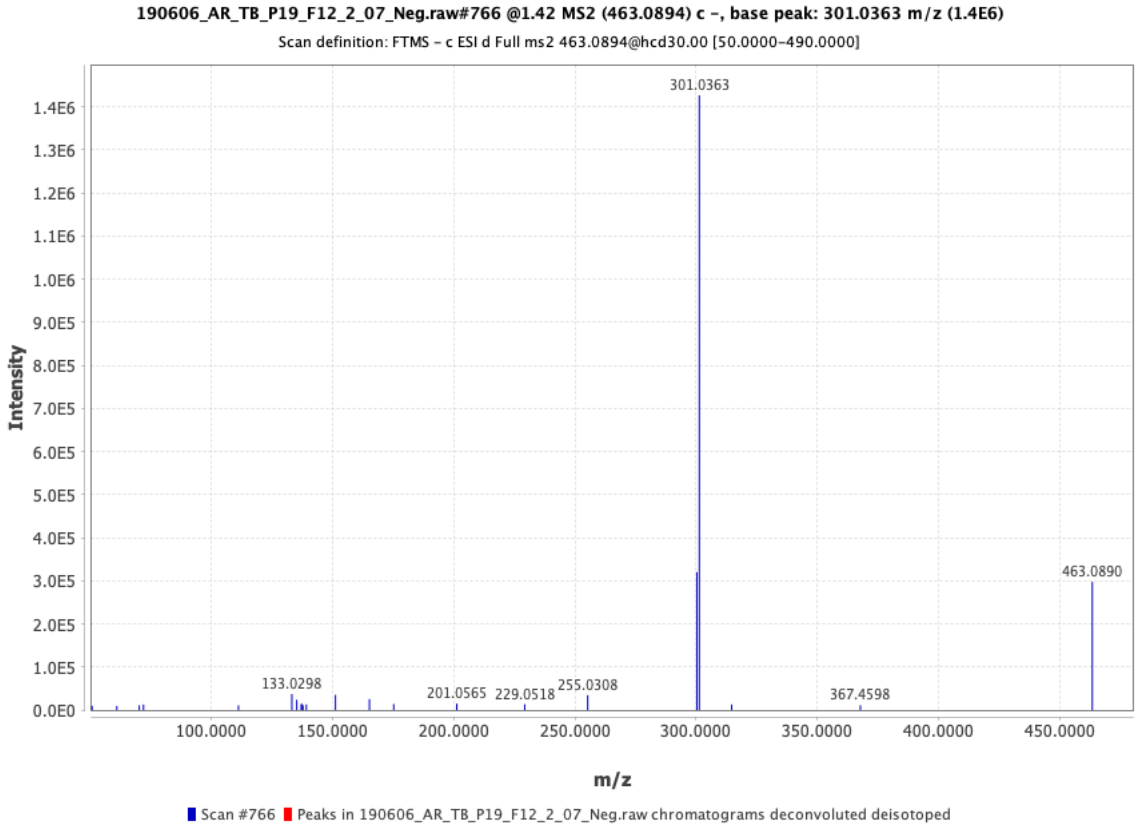
MS/MS spectra of compound **1** in the decoction of *N. lotus*.



MS/MS spectra of compound **7** in the decoction of *N. lotus*.

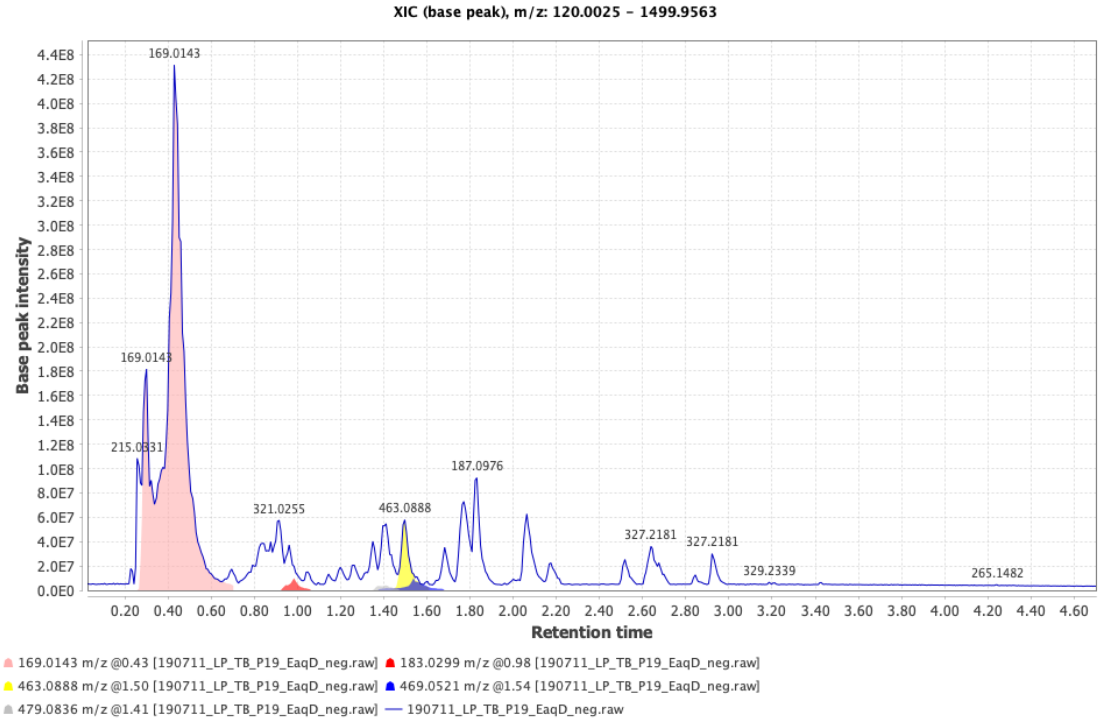


MS/MS spectra of compound **1**.

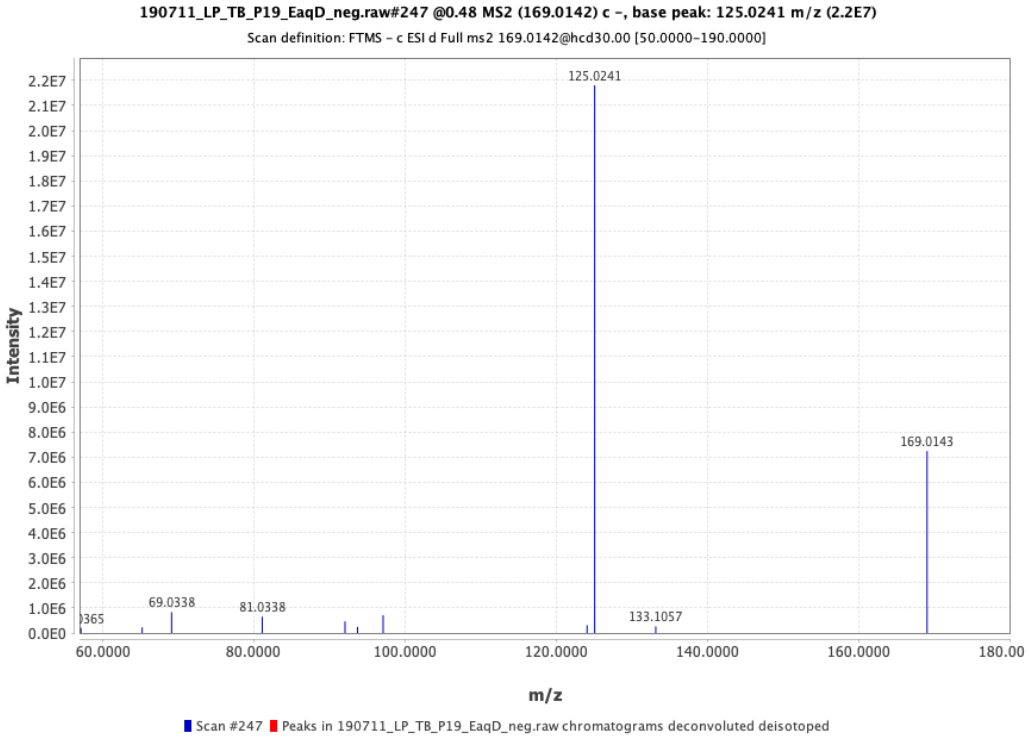


MS/MS spectra of compound **7**.

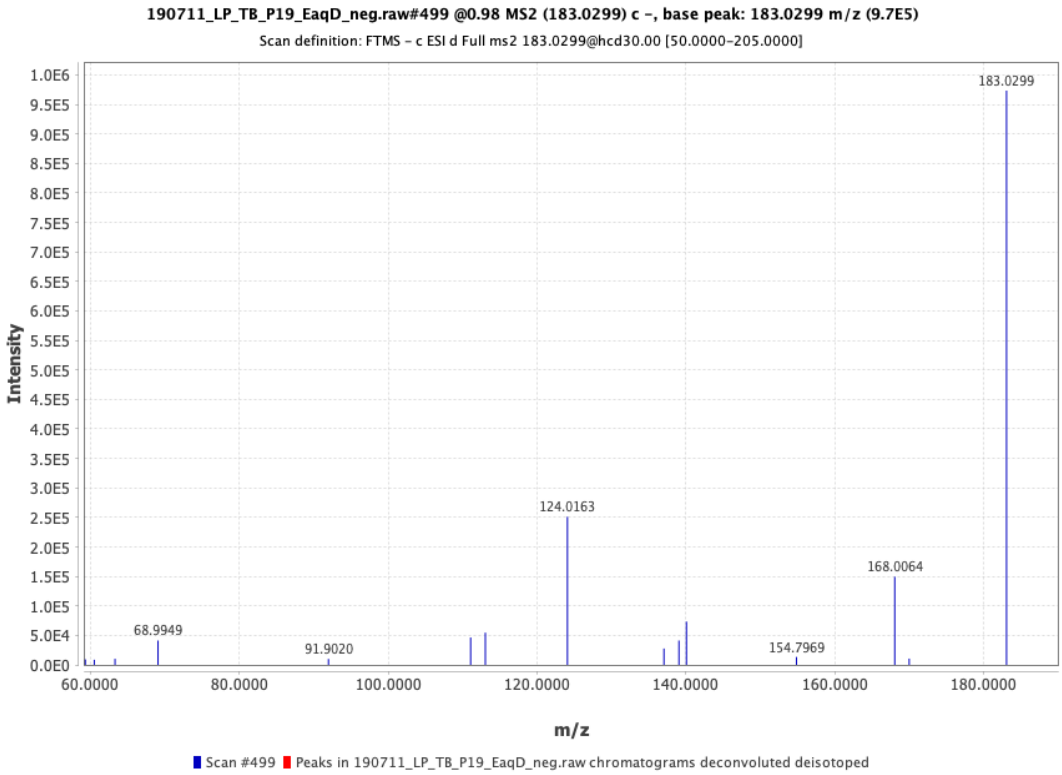
Figure S13: UHPLC-HRMS chromatogram showing the presence of compounds **1**, **2**, **5**, **6** and **7** in the decoction of *B. schreberi*.



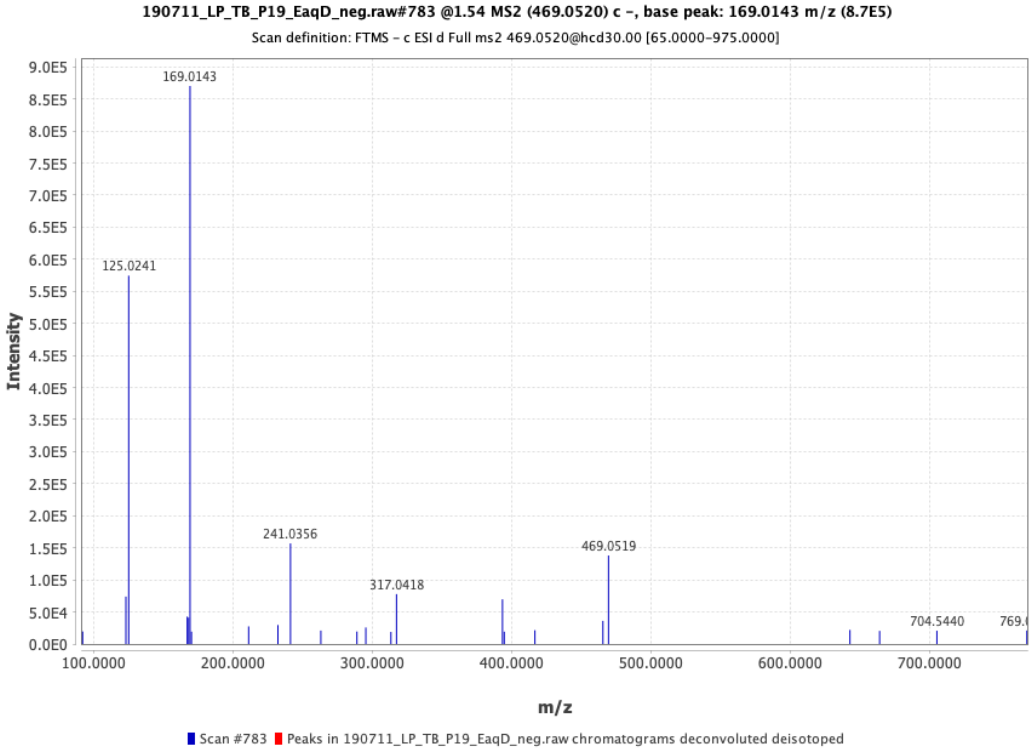
Chromatogram of the decoction of *B. schreberi* highlighting compounds **1, 2, 5, 6** and **7**.



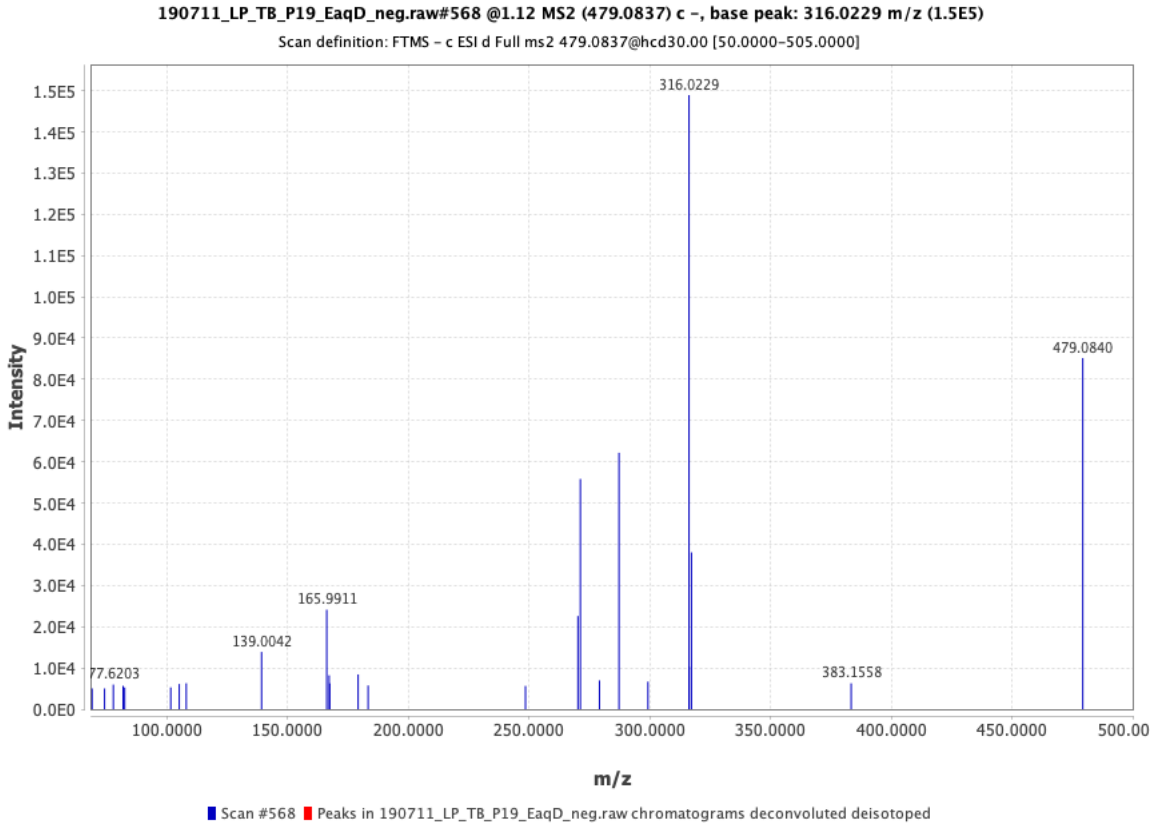
MS/MS spectra of compound **1** in the decoction of *B. schreberi*.



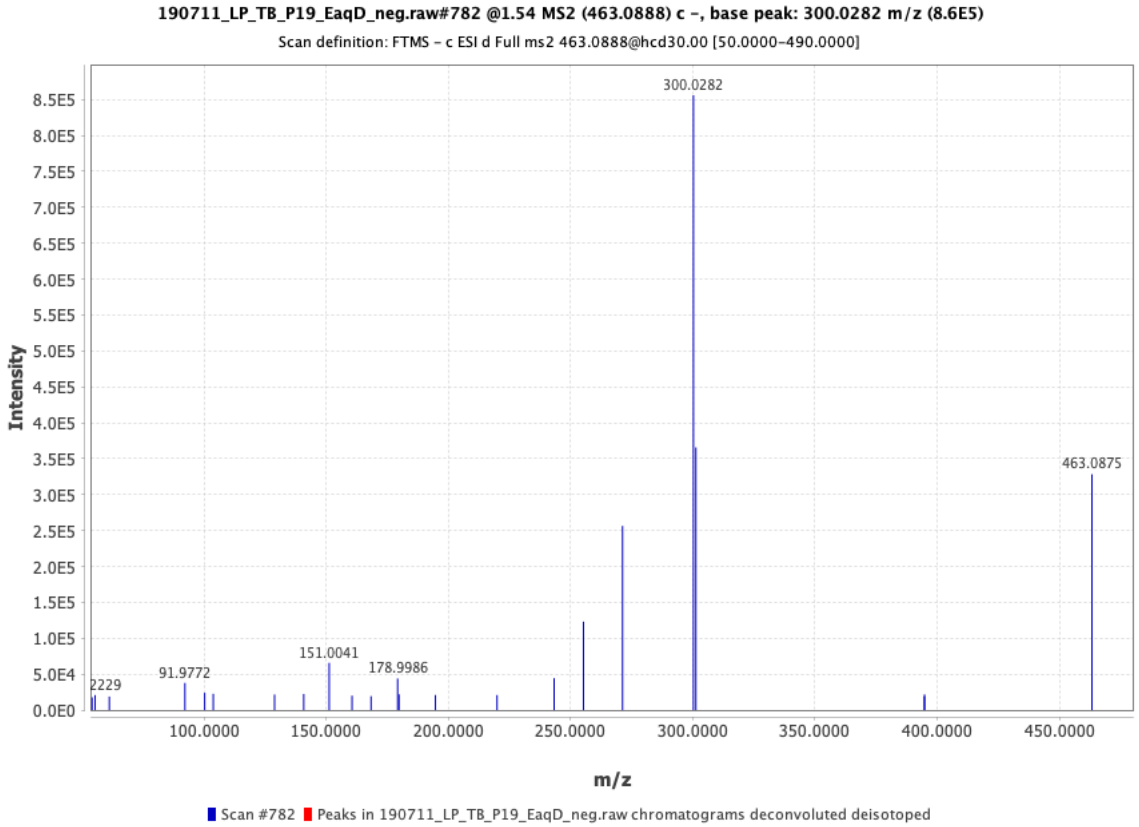
MS/MS spectra of compound **2** in the decoction of *B. schreberi*.



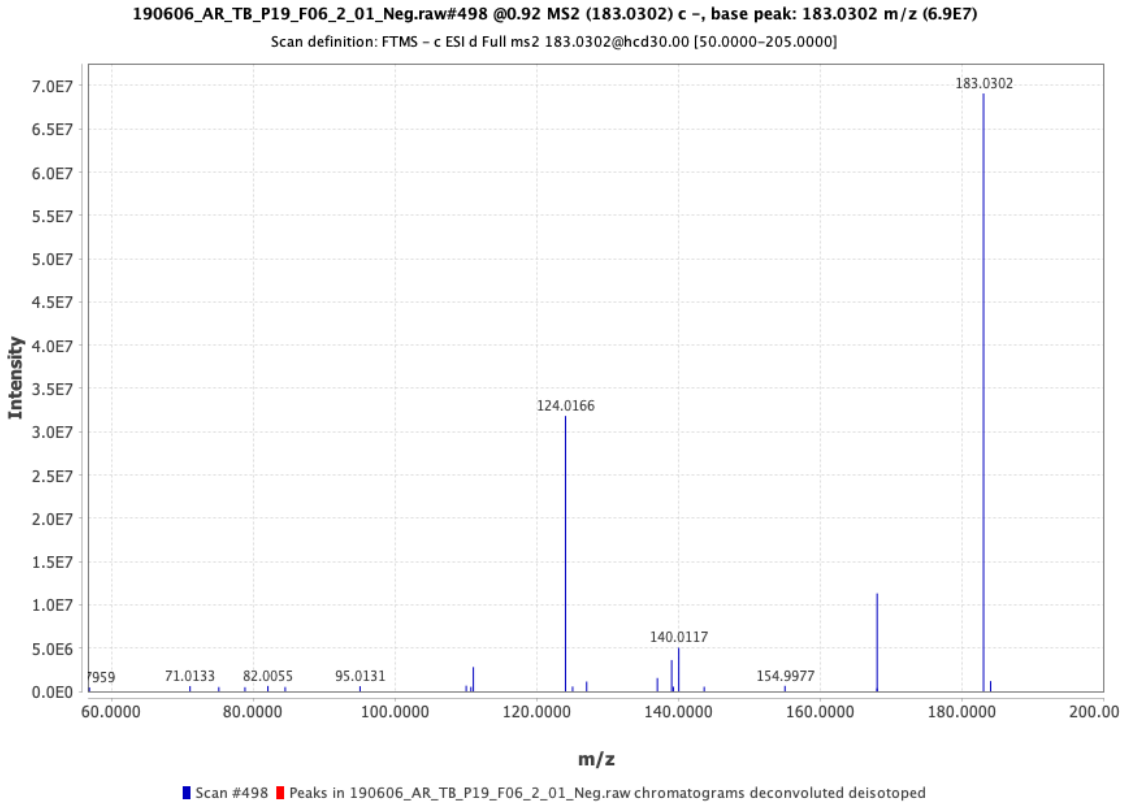
MS/MS spectra of compound **5** in the decoction of *B. schreberi*, only observed as *m/z* 469.0521 $[M-H]^{2-}$.



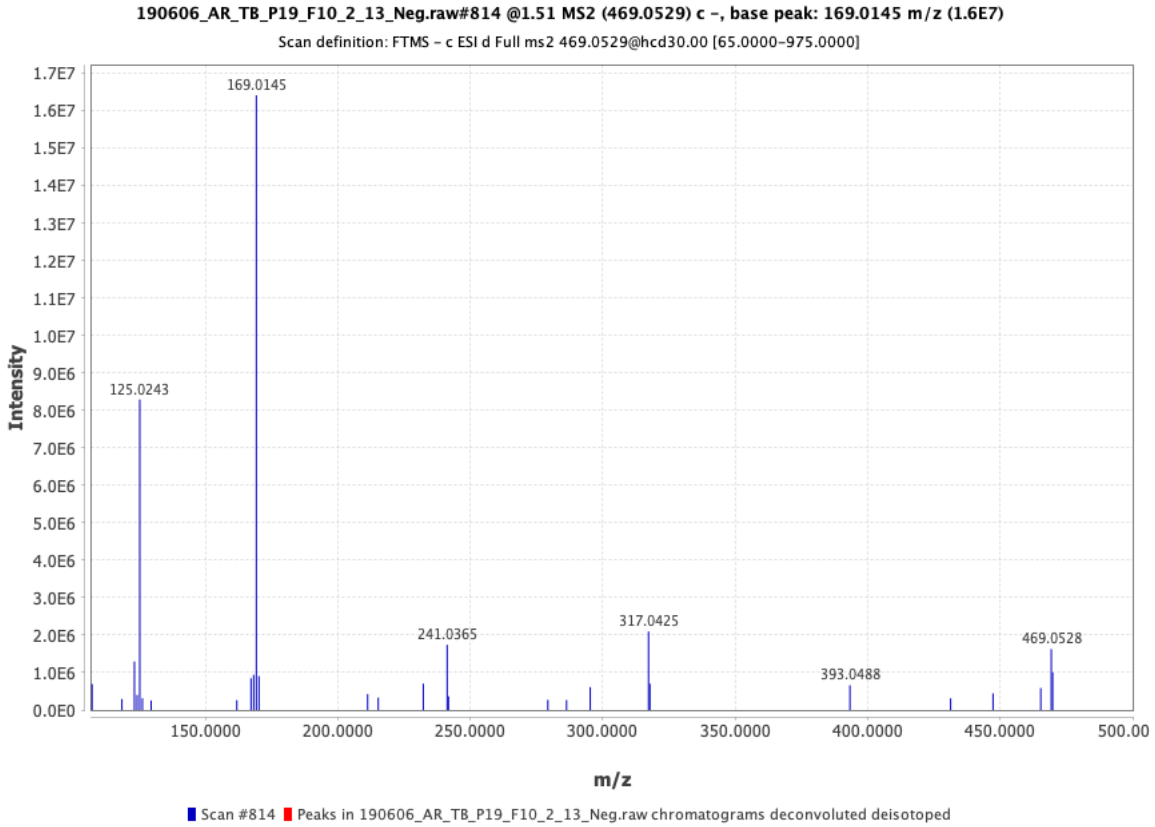
MS/MS spectra of compound **6** in the decoction of *B. schreberi.*



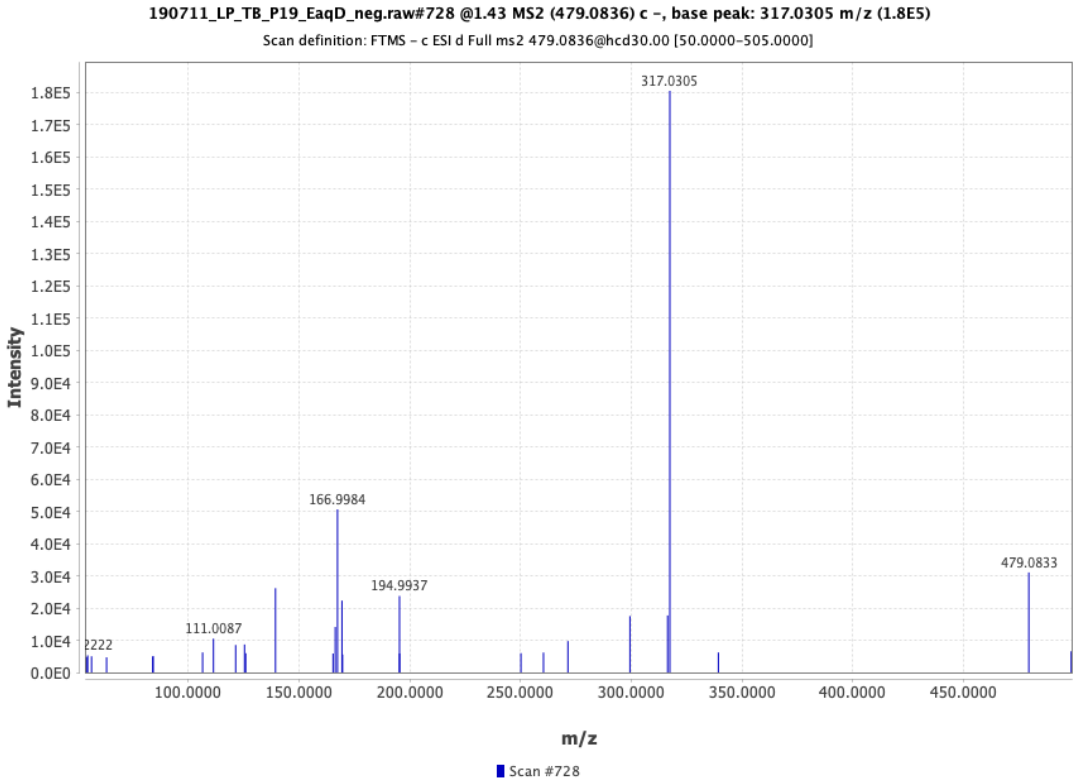
MS/MS spectra of compound **7** in the decoction of *B. schreberi.*



MS/MS spectra of compound **2**.



MS/MS spectra of compound **5**.



MS/MS spectra of compound **6**.

5 GENERAL DISCUSSION

5.1 Overview of the research outcomes

The valorization strategy of the Traditional Medicine launched by the WHO in the eighties has been followed by many African Member States and today, only few countries have not yet implemented a plan of action for the scientific validation of their traditional medicine. Angola is one of them, and with the aim of contributing to the valorization of the Angolan traditional medicinal knowledge, this research work was carried out.
As exposed in this work, the scientific validation process of traditional medicine is multifaceted, multidisciplinary and it brings together various skill sets. Therefore, it requires a strong network, mutual respect and trust among the different stakeholders.

Both colonial history and extended wars have challenged the Angolan scientific community in the advancement of research and more specifically in the ethnopharmacological research field. The absence of scientific data regarding ethnobotany and medical anthropology called for this multidisciplinary thesis work. Following the approach of the scientific validation of traditional remedies, this work aimed at (i) highlighting the access and the use to folk medicine in case of trypanosomiasis, (ii) reporting the plants used for this parasitic disease (iii) addressing risks of use related to some reported plants (iv) selecting the most promising crude extracts (v) identifying the active constituents in the selected plant extracts.
In the light of the above, the following major contributions of this work should be retained.

First, a procedural analysis of the complexity of national and international regulations concerning ethical, IPRs and biodiversity requirements was tackled. The implementation of the ethnobotanical study in a *Provider* country (Angola), which has not yet established ABS procedure, neither control measures to recognize the IPRs over the traditional knowledge raised compliance concerns with respect to these three aspects. In the absence of a legalized and regulated context, it was concluded that compliance can rather be achieved thanks to strong collaborative and personal advocacy efforts to respect three key ethical principles: transparency, fairness and mindfulness.

Second, the ethnomedical and ethnobotanical analysis of the use of herbal remedies against trypanosomiasis highlighted several key elements of the health seeking behavior and the botanicals used for this parasitic disease. The study revealed that among the infected persons, 40% turn to folk medicine before consulting a medical doctor. This significant adherence to herbal remedies could be explained by cultural reasons, family tradition and distance to health center. The ethnobotanical results pointed out 30 plant species used in the management of the disease, of which *Crossopteryx febrifuga* was the most cited plant. A literature analysis of the ethnobotanical practices across Africa showed that about one third (10 of 30) of the reported plants of our work could be correlated with the ethnomedicinal practice against sleeping sickness and its symptoms in other African countries. A comparative literature search on preclinical data pointed out that of the 30 reported species, about half (17 of 30) had their antitrypanosomal activity supported by in vitro and/or in vivo studies. Of this panel of 17

plants, three species *Entada abyssinica*, *Securidaca longipeduncunlata*, and *Ocimum gratissium* showed *in vitro* or/and *in vivo* evidence for the specific mode of preparation reported in our study. Three species, *Monodora myristica, Cymbopogon densiflorus,* and *Carica papaya*, haven't seen their traditional usage confirmed either by ethnobotanical surveys or by comparison with preclinical data. In addition, toxicity concerns were pointed out and some reported plants showed potential risk of use. As example, the use of the roots of *Aristolochia gigantea* in an aqueous herbal preparation raised serious concerns of the potential toxicity of this herbal preparation. Therefore, the importance of the feed-back session organized in phase 5, must be outlined. Indeed, this "feed-back" phase aims, amongst others, to report these concerns to the concerned population and discuss alternatives.

Pursuing the validation process of the Angolan traditional remedies used against trypanosomiasis, 9 species (of 30) were selected for further investigation, *Brasenia schreberi, Brillantaisia owariensis, Crossopteryx febrifuga, Entada abyssinica, Momordica charantia, Nymphaea lotus, Palisota schweinfurthii, Sarcocephalus latifolius,* and *Vitex madiensis.* The phyto-pharmacological and phytochemistry part of the thesis work calls attention to the following major points. After the bioguided-activity screening of 122 plant extracts, two crude extracts, the 80% ethanolic extract of *Brasenia schreberi* (leave) and the dichloromethane extract of *Nymphaea lotus* (leaf and leaflets) displayed an IC_{50} value < 10 µg/ml against *Trypanosoma brucei rhodesiense*. The bioguided-fractionation evidenced 7 active phenols for *B. schreberi* , namely gallic acid (1), methyl gallate (2), tetragalloylglucose (3), ethyl gallate (4), 1,2,3,4,6 pentagalloyl-β-glucopyranoside (5), gossypetin-7-O-glucopyranoside (6), hypolaetin-7-O-glucoside (7), and 1 active compound for *N. lotus*, an alkenyl resorcinol (8). Compounds (2-3, 5-6) were reported for the first time in the genus Brasenia and the presence of compound (8) wasn't so far described in the *Nymphaeacea.* It can be noted that the best antitrypanosomal activity against *Trypanosoma brucei rhodesiense* was found for gallic acid and ethyl gallate with an IC_{50} of 0.5 µg/ml and 0.6 µg/ml respectively. None of the compounds demonstrated interesting findings against *Trypanosoma cruzi*. The resorcinol alkyl showed against *Leishmania donovani* an encouraging activity (IC_{50}=2.5 µg/ml), however with a low selectivity (SI index= 5.2). Finally, in order to complete the scientific validation, the antitrypanosomal potential of the traditional preparation was assessed. We could evidence the presence of 3 active constituents in both decoctions: gallic acid, methyl gallate, and 1,2,3,4,6-Pentagalloyl-β-glucopyranoside. Gallic acid could be found in the largest amount, at a concentration of 50 mg /g and 21.804 mg/ g of extract for *B. schreberi* and *N. lotus* respectively.
It can be concluded that the phytochemical and pharmacological results provided primary evidence for the rational use of the decoction of *Brasenia schreberi* and *Nymphaea lotus* in the management of sleeping sickness in Angola.

This work has raised several questions: Is a fair and equitable sharing of the benefits in the ABS context realistic? How prioritizing plant candidates for further investigation? How could traditional medicine become a valuable support to modern medicine? Are there any other possible strategies to validate traditional knowledge?

After briefly discuss the idea of valorization promoted by the WHO (5.1.1), the next sub-chapters (5.2-5.5) discuss these questions.

5.1.1 Valorization strategy, a critical perspective

This research work illustrates the application of the valorization strategy put in place by the WHO.

The valorization of the TM as proposed by the WHO means the rationalization of traditional medicinal practices. However, the idea of valorizing the traditional medicine carries with it a paradox. What is fundamentally conceived through the idea of valorization, is to retain only knowledge and practices related to the plants. If these are "improved" by being validated, then they can play a role to provide health in a rational manner. In this sense, valorization means to bracket or reject the symbolic, religious, and magical attributes underlying this medicinal traditional system [296]. Thus, the promotion strategy of the Traditional Medicine and its scientific validation process have embodied a reductionist approach of the valorization and deprived the therapeutic practice from its cultural attribute. Therefore, from an emic perspective, the concept of valorization is questionable. Indeed, does ignoring the "symbolic and culturally-driven part" of a traditional therapeutic practice lead to a valorization of its knowledge? In regard to this question, Blé Marcel Yoro sees the role of the anthropologist as a possible key actor to "re-valorize" the holistic approach of the traditional therapeutic practices [297]. By delivering a qualitative analysis of the local therapeutic practices, the anthropologist account for the perceptions and understandings underlying the practice of traditional medicine. Though an adequate argumentation of this point is beyond the scope of this sub-chapter, I would suggest that anthropologists should always be integrated within a scientific team commit to assess and valorize local remedies and practices.

5.2 Is a fair and equitable sharing of the benefits realistic?

Regarding the procedural aspect exposed in chapter 2, one main challenge is the application of the ABS measures. The fundamental moral principle, on which rely the CBD and the NP, is the concept of fairness and equity. However, surprisingly, nor in the CBD neither in the NP, there is a clear definition of this moral foundation upon which relies the application of the NP [298]. This conceptual lack pushed Schroeder (2006) to have provided a definition: benefit-sharing is described as "*the action of giving a portion of advantages or profits derived from the use of genetic resources or traditional knowledge to resource providers in orders to achieve justice in exchange"* [299]. Based on that, one can ask oneself: what "portion of advantages or profits" could be considered as a fair and equitable sharing? From own experience, I would argue that the just share can only be determined on a case-by- case basis, dependent on the contractual agreement between parties. More specifically, the nature of the benefits to be compensated has to be submitted to negotiation between the parties. In case of basic research, as it was the case in this work, monetary benefits are not contemplated, because the scientific activity does not provide a direct return on the investments.

Considering the in-kind compensation, different non-pecuniary compensations can be proposed depending on the case. For example, Berg (2001) proposed that in the context of

clinical trial, the in-kind benefit could be a free medical assistance to participating communities in the research project. If we transpose this proposal to the context of bioprospection, the benefit could be free access to the derived knowledge and drug for the providing communities. This model of benefit-sharing is known as “reasonable availability” [300]. Accordingly, the benefits for the providing parties will be the access to the findings of the research and its derived product(s). Though this case can only be applied to research projects that generate, after many years, tangible products, nevertheless this kind of compensation could contribute to knowledge and health empowerment of the providing, often poor, local communities. The second model is the “fair benefits” model [301]; it argues that benefits should not strictly be bind to the results of the research, but that compensations could be of other forms and not necessarily emerged from the research’s findings. Whatever may be the chosen model, they both seem to turn the in-kind compensation as a contribution to a socio-economic upliftment of impoverished communities. These two models, support the idea that a “fair and equitable sharing” stems from the "portion of advantages or profits" granted to the *providing* communities.

I would rather align to Bachman (2011), which supports the idea that “fair and equitable sharing” does not stem from the "portion of advantages or profits" granted to the communities but resides in the agreement procedure itself.
In the absence of substantive criteria of fairness and equity within the Nagoya Protocol, what can guarantee to be fair and just is the negotiation on itself. Indeed, the fact that both parties have a moral obligation, as contracting parties of the Nagoya Protocol and more specifically through the binding agreement of the MAT, to comply with the normative rules, should guaranty that they will show due diligence in negotiating the benefit-sharing agreement in a fair and transparent way. Based on this moral sense, it is the parties responsibility to conduct transparent negotiations and to determine the benefit to be shared as well as to agree on its fairness. As so, one could admit that what is qualified as fair and equitable in the agreement terms is neither the quantitative nor the qualitative value of the counterpart itself but rather the procedural aspect of the negotiation[28]. According to this argument, and based on own experience, one of the most challenging aspects to ensure a fair negotiation is that all concerned persons should have sound knowledge to understand the scientific and legal issues related to the research project to be agreed upon. Lacking of in- depth knowledge and adequate skills of understanding the challenges posed by the research project could end in a power relationship between parties, where the asymmetric repartition of knowledge between *Users* (often private biotechnological or academic actors) and *Providers* (often local and indigenous communities) could prevent the less competent to make an informed and conscious decision while negotiating the terms of the agreement [302].

If the negotiation procedure is the place of a fair and equitable exchange, then I propose to consider the establishment of the MAT as a transdisciplinary process, in which all stakeholders are potential (in)direct beneficiaries of the research results. By doing so, all participants would

[28] Bachmann, A. (2011)

jointly frame, analyze, elaborate in details and agree on what should be the content of the benefices at stakeholder level as well as at project level. In this context, I see the occurrence of the MAT as a round table, which would bring together all actors from the very beginning of the project and would enable interactive knowledge production. The transdisciplinary approach enables, thus, that the different type of benefits arising from the project and corresponding to each stakeholder's perspective can be defined, so that none of the expectation of the parties would be a stand-alone. Consequently, the establishment of a MAT from the beginning of the project as a transdisciplinary process can be a means of producing a new understanding of the benefits at stake and generating a co-produced knowledge for e.g on the use of botanicals, on the conservation of herbal medicines, on the role of herbal medicine in local health dynamics, or on local commercialization of herbal remedies, etc.

Such a transdisciplinary process would not end in a "fair and equitable" sharing of the benefits but rather in a "useful and desired" sharing of the benefits, what sounds (to me) closer to the expectations of the requesting parties.
Nevertheless, one critical point by pursuing transdisciplinary approach in an ethnopharmcological project, is to secure funding due to a more important time-to-completion. Indeed, by integrating from the beginning academic, non-academic, private/public stakeholders and proceeding to the mutual learning and co-production of knowledge needs a longer span of time to bring to fruition the project.

Though this transdisciplinary approach could not be applied in the framework of this work, at the time of the negotiation of the MAT, nevertheless the phase 5 of the project (see chapter 2) aims at being an avenue for a transdisciplinary workshop. Indeed, during this feed-back session all stakeholders will be invited in order to exchange about the scientific findings of this work but also to share the different understandings about the role of herbal remedies in the public health system. By recognizing and valuing local knowledge on herbal remedies, phase 5 will discuss the practical implications of the research project and the benefits at stakeholders' level, for example the elaboration of a booklet on potential toxicological plants or the creation of a medicinal garden at house level.

5.3 How prioritizing plant candidates for further investigation?

The selection of the plant candidates for further investigation is a key step in the validation process. Data gathered through ethnobotanical studies and literature searches provide information, which can serve as pre-screen to select the plants for further pharmacological investigations. However, in the ethnopharmacological field, it is rather unlikely that the selection criteria are discussed and standardized. Two ranking systems have been developed by the group of the *Research Initiative on Traditional Antimalarial Method* (RITAM) in the context of the validation of traditional antimalarials. The first one, is a scoring system, called the "*Important Value for the treatment of Malaria*" (IVmal) [303]. The litterature-based ethnobotanical information for a specific plant species is assigned with an "IVmal", according to the geographical dispersion of its use. Thus, a plant species reported to be used against

malaria in two different continents will receive a better scoring than a plant that has been reported only in two different countries on the same continent. The hypothesis underlying this scoring method is that: the more widespread the use of a plant species, the more it has the potential to have antimalarial properties. In this system, nine grades have been determined. As so, the highest grade is the "IVmal" equal to 8, which means that the plant species has been reported in three continents. The lowest grade is the "IVmal" equal to "?", which refers to plant species for who no sufficient data are available. Based on an extensive literature comprising 94 original ethnobotanical publications and conducted in 33 countries, the RITAM applied this scoring method, and they could identify 11 species with an "IVmal" value equal to 8. Thus, these 11 plants were selected further for pharmacological investigations. The RITAM scoring method improves in that sense the widely employed ethnobotanical index, "frequency of citation" or "frequency of quotation" [304]. However, one limitation of this scoring method is, that the disease of interest has to be widely dispersed, and it would not be possible to apply it for trypanosomiasis, without prior adaptation to its geographical distribution.
Another ranking system for prioritizing the plant candidates to enter the screening process is the RITAM score [305]. It is an overall score composed of 3 components for each remedy of interest: (i) the frequency of citation in ethnobotanical studies, (2) the efficacy *in vitro* and *in* vivo (iii) the safety of the studied remedies. The RITAM score was assessed by correlating it with the results of clinical trials of antimalarials remedies. And it was concluded, that this scoring system can be used as part of the selection process for prioritizing plants for further research as anti-malarial drug candidates.

For the purpose of this thesis work, a selection grid was elaborated in order to select 8 plants out of the 30 reported botanicals described in chapter 4.
Four inclusive criteria and one exclusive criterion were applied. The inclusive criteria were:

1. The Use Report index (UR)
2. The correlation with preclinical data
3. The quality of the narrative content
4. The novelty

The Use Report index. Every plant usage reported in a herbal recipe during the ethnobotanical study was considered as a separate record and counted as one use report (UR) [98]. If a plant species was mentioned several times in different recipes, the use report was accordingly counted. Based on the Use Report index, all plants species quoted with an UR lying between 6 and 2 were preselected (see Table 4-3, chapter 3). This first selection parameter retained 8 plants of the 30 reported species, *Crossopteryx febrifuga*, *Vitex madiensis*, *Palisota schweinfurthii*, *Momordica charantia*, *Entada abyssinica*, *Sarcocephalus longipedunculata*, and *Ocimum gratissimum*.

The correlation with preclinical data. Of 30 species, 17 (56%) had their antitrypanosomal activity supported by in vitro and/or in vivo studies (see Table 3-5, chapter 3). Based on a correlation between the traditionally reported herbal preparation (most often a decoction)

and the *in vivo* clinical results of the extracts that mimicked most closely the traditional preparation were retained (aqueous extracts). This second parameter enabled selecting five species, *Nymphaea lotus*, *Senna occidentalis*, *Securidaca longipedunculata*, and *Ocimum gratissimum*.

The quality of the narrative content. This third selection parameter refers to the qualitative appreciation of the information entrusted during the interview with the traditional practitioner. The qualitative appreciation was based on: (i) level of a detailed information, (ii) a same response to a repeated question, (iii) the veracity of the facts, (iv) a collaborative attitude. Based on this parameter, one species was retained, *Nymphaea lotus*.

The novelty. This last criterion aims at selecting reported plant species with an UR ≥ 2, for which none literature reference for the disease could be found. This last criterion enabled selecting two species, *Brillantaisia owariensis* and *Momordica charantia*.

One exclusive criterion was applied. This was that any plant reported by the lay people (patients) were discarded. This was justified by two reasons: (i) the knowledge of the medicinal plants used to treat HAT in Angola was analyzed as a "specialized knowledge belonging to specialists' group rather to lay people"; (ii) some of the interviews with the patients had been performed by the students and not by the PI, thus the third selection parameter would not have been fulfilled. Applying this exclusive criterion, the following plants were discarded: *Ocimum gratissimum, Senna occidentalis*.

Based on these criteria, the following 8 plant species should have been retained, *C. febrifuga*, *V. madiensis*, *P. schweinfurthii*, *M. charantia*, *E. abyssinica*, *S. longipedunculata*, *N. lotus*, *B. owariensis*,. However, due to time and logistical constraints, one species, *Securidaca longipedunculata*, could not be collected in large amount for further investigations. It was decided to be replaced by *Sarcocephalus latifolius*.

In addition to the inclusion and exclusion criteria, a first check of the toxicity profile of the plant candidates was always undertaken in order to discard, if needed, a plant candidate.

However, it must be underlined that combining the different components for selection of promising species can also lead to mismatches. As was observed in the case of the RITAM score, *Cinchona* (the highly effective plant against malaria and source of quinine) obtained a low overall score, which would have led to discard this species as a plant candidate against malaria [305].
One limitation of this selection method is the paucity of *in vivo* clinical studies on antitrypanosomal medicinal plants. Indeed, the correlation between the traditionally reported herbal preparation and the preclinical data has been demonstrated to be a useful predictor of clinical efficacy [305]. Based on this, *Nymphaea lotus*, *Senna occidentalis*, *Securidaca longipedunculata*, and *Ocimum gratissimum* should be tested *in vivo* for their potential as a traditional antitrypanosomal remedy. To this point, the fact that *Senna occidentalis* and

Ocimum gratissimum were discarded (because patients reported them) is a critical point of this selection method used in this work.
This selection grid could be improved in future, by taken into account how extensively the plant is used across its distribution in trypanosomiasis endemic regions.

As the selection of the plant candidate is a crucial aspect in the validation process, it would be advisable that the ethnopharmacological studies explain more thoroughly the selection process used to retain the tested plant candidates. It would bring more transparency in the selection process and enable to develop in future, a "standardized" grid of selection for a targeted disease, as was elaborated by RITAM.
In the absence of standardized selection criteria to prioritize the promising plant candidates for further selection, the choice of the plant of interest remains quite subjective.

5.4 How can traditional medicine be a supportive therapeutic instrument to modern medicine?

It is believed that the current assumption of "*one drug can fit to all"* will be unsustainable in future [306].
In chapter 3, the ethnobotanical study revealed that even though a conventional drug was delivered free, 40% of the involved people recourse to herbal medicine before having received the referenced treatment. One of the reasons, which prevented the patients to access the conventional drug was the long distance to the health center. Indeed, living in remote areas often complicates the access to the available conventional treatment. Having a validated traditional medicine could serve as an alternative treatment and act as a first-aid treatment for a rapid care, until the sufferer can consult a medical doctor [32].
In the case of trypanosomiasis, except cynaropicrin [54], there is, to the best of our knowledge, so far no potential plant candidate proving efficacy in inhibiting the development of the parasite. Traditional remedies could rather be used as a complement medicine to the classical treatment. The herbal remedy could be used to alleviate or reduce one or many of the clinical symptoms accompanying the course of the disease, such as anemia, intermittent fever, hepatomegaly, jaundice and immunosuppression. As example, an aqueous extract of *Scoparia dulcis*, was tested in rabbits infected by *T. brucei*. The results showed that treatment with *S. dulcis* at a daily oral dose of 25 mg/ Kg body weight significantly reduce the severity of the trypanosome-induced immunosuppression, when compared with untreated infected animals. *S. dulcis* was thus considered, as a potential plant candidate in protecting against the parasite-induced decrease in the population of immunologically active cells [307] .
As clinical recovery and parasite clearance depend not only on the efficacy of the remedy but also on the level of immunity of the patient, therefore immunostimulating herbal remedies could be an interesting complementary medicine to the conventional drug in endemic areas.

Several studies investigated the role of the herbal remedies as a complement therapy in case of HIV/AIDS [308-310]. In that sense, it would be interesting to investigate if, the selected traditional herbal preparation made of *B. schreberi* and *N. lotus* could act as a complement

therapy in case of trypanosomiasis by alleviation or reduction of one or many of the clinical symptoms accompanying the course of the disease.
Another very interesting aspect that should draw attention is the use of herbal remedies in case of resistance or multi-resistance. Indeed, as an herbal preparation is made of dozen of constituents, it can be considered in itself as a combination therapy. Thus, combining a validated herbal medicine to a conventional drug to prevent development of drug resistance phenomenon is an area of investigation to consider, moreover in endemic region with a high level of transmission. It has already been explored in case of antimicrobial resistance [311]. For instance the different ethanolic extracts of propolis, showed synergism with ampicillin, ceftriaxone, and doxycycline for multidrug-resistant *S. aureus* [312]. Another example is given by the tea extract of *Camellia sinensis,* and several of its components, which reverse the resistance to beta-lactams [313, 314]. However, the screening method of a combined phytomedicine with a synthetic drug is demanding and complex in order to quantify the synergism and assess the adverse effects [315].
The combination of a validated herbal remedy to a conventional drug could have the advantage of extending the use of a conventional drug, before emergence of drug resistance phenomenon, and contributing to the control of the resistance development [316].

5.5 Classical drug discovery screening: is there any other possible strategy to validate traditional medicine?

The scientific validation process of traditional herbal remedies relies largely on the classical pharmacology approach for the discovery of new chemical entities. Thus, after the selection of the herbal remedy of interest, the plant extract will only be tested *in vivo*, after *in vitro* activity screening and full characterization. Thus, the scientific assessment of the plant extract will consist in extracts' activity screening followed by the isolation, identification, and structure characterization of the active constituent(s). The most promising extract will only go further for *in vivo* testing, if the selection criteria as for example IC_{50} values, selectivity index are met. The drawback of this approach is that interesting plant extracts, made of dozen of constituents, are discarded due to the stringent selection criteria normally used in the drug discovery process applied for a single active compound. Indeed, the active constituents may be in insufficient quantities in the crude extracts to display an activity at the tested concentration. It must be mentioned, that the same prevails for cytotoxic evaluation of the crude extract. Alternatively, the presence in high quantities of an active constituent in the crude extract could be counteracted by other antagonistic ingredients. On the contrary, a synergistic effect of several active compounds in the crude extract can enhance the activity at extract level, and induce loss of activity at fraction level. As example, the bioassay-guided isolation of the diastereosiomer of kolavenol from *Entada* abyssinica showed that the IC_{50} value of this isolated constituent was less favourable than the activity value of the fraction from which it was isolated [184]. Luckily, in this work, the fractionation procedure of the crude extracts of *B. schreberi* and *N. lotus* did not result in loss of activity, on the contrary, the crude extracts displayed, in both cases, a lower growth inhibition (GI) value (respectively GI%=84.6 and

GI%=81.2) than the selected active fractions (for *B.schreberi* GI% F3: 99.4; GI% F6: 98.2; GI% F10: 95.1; GI% F11: 94.7; GI% F12: 97 and for *N.lotus* GI% F4: 97.4).

From the above, we can see that the selection of an active crude extract is quite challenging and several other limitations related to the standard drug discovery process can be added to the first ones.

First, one has to remember that the plant extracts are mainly prepared from the dried plant material. However, the traditional practitioner, specifically those located in remote areas, prepare their herbal remedies from fresh plant material. Thus, there is a possible loss of active constituents, which are only present in the fresh plants, as for example essential oils.

Second, the collection step of the plant material is a limiting factor. Indeed, very often, it is complicated to follow the exact traditional collection procedure, when collecting a large amount of plant material. Most of the time, the traditional recipe includes specific seasonality of collection, phenotype of the plant, state of maturation, environmental ecological characteristics, etc. These different parameters influence the intrinsic characteristic of the quality of the starting material for the production of the plant extracts, and can lead to variation or loss of activity, when testing different batches of the same plant species. The relation between the vegetative stage of the plant and both the antitrypanosomal and antiplasmodial activities as well as the cytotoxicity was investigated in a study conducted with crude extracts and essential oils of *Ocimum gratissimum* from Benin [161]. The study evidenced that the activity varies according to the vegetative stage (pre and full flowering) and the plant part (seeds, stems and leaves) extracted. This phenomenon was observed in our study with the three varieties of *Nymphaea lotus*, for which two varieties (extract ID 90 /*N. lotus* and extract ID 114/*N. lotus* Bungo batch) displayed a moderate antitrypanosomal activity (GI% ranging between 51-70) and one variety (extract ID 121 / *N. lotus* Damba batch) was inactive (GI%= 18; see chapter 4, supplementary material, Table S1). Consequently, the composition of the plant extract to be tested *in vitro*, depends on these ecological and climatic parameters, thus the concentration of the active constituents can vary among in all samples or even not be present. Hence, mimicking the most closely a traditionally used herbal preparation seems very challenging.

Though the quality of the plant material could not be assessed in the framework of this validation process, it is crucial to underline, that a quality assessment of the raw plant material under investigation has to be carried out, parallel to or at the latest after activity and toxicity profiling of the plant candidate [317]. Using high performance thin layer chromatography method (HPTLC), comparison of the chemical profiles between the tested plant extracts can be done. Thus, an initial fingerprint of the active extracts as well as of the subsequent active fractions should be performed, in order to compare the phytochemical profiles of the extracts of interest. As so, the major changes in chemical composition can be correlated to a change in activity.

Another limitation factor of the standard drug discovery procedure is that there can be activity discrepancies between *in vitro* and *in vivo* results. A good *in vitro* activity does not necessarily result in a good *in vivo* activity. Conversely, inactive extracts or constituents can turn to be active *in vivo*, due to metabolism. Even though, this is an inherent problem of the preclinical procedure, it is even more marked in the case of a plant extract, made dozen of constituents.

In addition to these methodological concerns, a question remains: "what is a meaningful concentration to test a crude extract at, in order to get a meaningful result? In other words, at which tested concentration should a crude extract be assessed in order that the resulting activity can be a reliable selection criteria? As example, in the in-house screening procedure at the PCU the tested products (plant extracts or single synthetic compounds) can be retained for further investigations, if the IC_{50} value ≤ 1µg/ml. However, at extract level, is an IC_{50} value of 1 µg /ml adapted in order to validate an active extract? And if so, what should be the initial concentration for a plant extract, 5, 10, 20, or 50 µg/ml? These questions underline, that relating to herbal medicine validation, there are no standards regarding the tested concentration, as is the case at industry level [318]. Therefore, standard criteria should be developed for the evaluation of plant activity in order to make comparison between the different studies.

Considering all these challenging and critical aspects, another approach has been proposed to validate herbal remedies, the reverse pharmacology [306]. Reverse pharmacology is a transdisciplinary approach combining traditional knowledge, experimental observations and clinical outcomes [319]. This approach reverses the classical drug discovery process "from laboratory to clinical "and proposes "from clinical to laboratory". Hence, it enables to select the most promising traditional remedy, by first assessing its clinical effectiveness in human with primary observation. The prerequisite of this approach is to have access to many clinical observations in order to generate sufficient pharmacoepidemiological data, which can evidence the safety and efficacy of the herbal medicine under investigation. This can be examplified by the Ayurvedic texts, which abound in polyherbal formulations and can serve as primary source for the validation of Ayurvedic preparations. The best case of a successful story of the reverse pharmacology is illustrated by the discovery of artemisinin.
Reverse pharmacology applied for the scientific validation of traditional remedies takes advantage of the experience and practices on medicinal plants build over centuries by local communities to treat a certain disease in a specific area. Consequently, this approach evaluates herbal preparations already used by humans, on the contrary of the drug discovery process, which aims to discover a new (or re-discover an old) active compound, not yet used for a specific disease.
The retrospective treatment outcome study (RTO-study) is in line with the reverse pharmacology-based approach, and was proposed in the frame of the search for a new antimalarial traditional medicine [320]. The RTO-study takes advantage of the fact that traditional remedies are in use by local communities, thus generating clinical observations. This ethnomedical survey technique enables to retain the most promising herbal treatments by correlating patients' recovery and herbal treatment used. Thus, by providing indices of effectiveness and safety about traditional treatments used, the RTO-study provides a panel of best clinically active plant candidates for further analysis. An example of a successful application of the reverse pharmacology approach in validating a traditional medicine is given by *Argemone mexicana* [320, 321]. However, it has to be underlined that the reverse pharmacology approach is dependent on a large amount of available and accessible clinical

observations. Therefore, because of the low incidence rate of trypanosomiasis in Angola, such a study design would not have be applicable. An ideal targeted parasitic disease, which is endemic in Angola and more suitable for this approach, is schistosomiasis. Hence, for future investigation and scientific assessment of the Angolan traditional medicine, it would be suitable to choose schistosomiasis and start the validation process with an RTO-study.
One crucial difference in the targeted objective between the classical drug discovery process of a lead compound and the validation process of traditional remedies is that in the latter case, the scientific assessment has to confirm, the validity of a longstanding therapeutic practice used by hundreds or thousands of patients. Therefore, what matters first is the safety profile of the herbal remedy of interest rather than its efficacy. There is little doubt that a traditional remedy can stand the test of time, if it induces immediate side effects, as in case of an acute toxicity. On that topic the WHO guidelines state that: "*If the product has been traditionally used without demonstrated harm, no specific restrictive regulatory action should be undertaken unless new evidence demands a revised risk-benefit assessment*" [322]. However, long-term toxicity is yet more difficult to correlate with the intake of an herbal remedy. Very few studies on the use of herbal remedies report adverse events. Consequently, more should be investigated on the toxicological profile of the traditional remedies. In this sense, the traditional practitioners can have a key role in reporting any observed or mentioned side effects due to a prescribed herbal treatment. Assessing the risk associated to the herbal treatment from the start of the validation process will contribute to build up a pharmacovigilance system of the herbal remedies, which are used locally for a given affection. This would enable to unmask mid- and long-term adverse effects related to the prescribed remedies, a task which is under-investigated in the field of the validation of traditional remedies.

5.6 Limitations and perspectives

A general constraint encountered in the validation process of herbal remedies, is the non-disclosure of the specific mode of preparation of the herbal remedy. This situation is more likely to happen, when working with specialists (traditional practitioners) than with lay people (local community). Indeed, during the interviews led among the traditional practitioners, twice the interview had to be interrupted, because of the secrecy of the mode of preparation of the herbal recipe. Moreover, as a scientist having signed a non-disclosure clause and willing to respect the IPRs of the holders of the traditional knowledge, I was not in a position to reveal the exact mode of preparation in the published data. This aspect is highlighted in table 3-2 of the Chapter 3. The mode of preparation of the remedy is given in a general way, without specific information, as for example on the dosage form.
In the context of research development of the TM, this ambiguous situation compromises the replication, comparison and eventual improvement of the mode of preparation of the remedy. An example is given with a clinical trial led in Uganda, which aimed at evaluating the effectiveness of an herbal remedy called "AM". Imprecise information of the herbal recipe, led to the administration of an non homogeneous herbal product along the study [33].

Trypanosomiasis was chosen as the disease of interest on recommendation of former Angolan Minister of Health. However, on major constraint is that the fact that it is a life threatened disease, the robustness of a validated traditional remedy has to be strong. In addition, the actual low incidence, make it impossible to perform a RTO-study. However, this work is a first contribution to the scientific validation of an Angolan traditional remedy, and it can be used in future as a prime experience. Taking in account the high prevalence of schistosomiasis in Angola, I would suggest that this endemic parasitic disease could be a good target to initiate the validation of traditional medicine to treat this affection.

Based on the outcomes of the ethnopharmcological literature analysis (see Table 3-5/ Chapter 3), it is recommended that *Nymphaea lotus*, *Securidaca longipedunculata*, *Entada abyssinica*, *Ocimum gratissimum* and *Azadirachta indica should* have their traditional preparation be tested *in vitro* or/and *in vivo* to evidence their potential as a traditional antitrypanosomal remedy.

From the results of Chapter 4, the traditionally used decoction made of *N. lotus* and *B. schreberi* should be tested both plant extracts together *in vitro* and *in vivo*. However, their exact ratio is not known, and different mixtures of these two plants should be tested.

5.7 Final conclusion

This work enabled to (i) confirm the use of herbal remedies in the management of trypanosomiasis in Angola, (ii) select two traditionally used medicinal plants, *Brasenia schreberi* and *Nymphaea lotus*, and evidence their antitrypanosomal activity by the identification of active constituents. The presence of some of the active identified constituents could be confirmed in the traditional preparation (decoction). As such, this thesis work is a contribution to the scientific validation of a traditionally used remedy in the management of trypanosomiasis in Angola.
Additionally, this work serves as a case study for questions related to ethical, IPRs and ABS requirements in the context of access to genetic resources and associated Traditional Knowledge. Thanks to a successful collaboration between the Swiss TPH and the different Angolan stakeholders, and particularly the CNIC and the INBAC, Angola has issued its first *Mutually Agreed Terms* (MAT) with a Swiss research entity.

The encouraging outcomes of this research work will hopefully contribute to triggering the validation process of the Angolan herbal remedies.

References

1. Büscher, P., G. Cecchi, V. Jamonneau, and G. Priotto, *Human African trypanosomiasis.* The Lancet, 2017. **390**(10110): p. 2397-2409.
2. Brun, R., J. Blum, F. Chappuis, and C. Burri, *Human african trypanosomiasis.* The Lancet, 2010. **375**(9709): p. 148-159.
3. Checchi, F., et al., *Estimates of the duration of the early and late stage of gambiense sleeping sickness.* 2008. **8**(1): p. 16.
4. Odiit, M., F. Kansiime, and J.J.E.A.m.j. Enyaru, *Duration of symptoms and case fatality of sleeping sickness caused by Trypanosoma brucei rhodesiense in Tororo, Uganda.* 1997. **74**(12): p. 792-795.
5. World Health Organization. *WHO interim guidelines for the treatment of gambiense human African trypanosomiasis. World Health Organization.* 2019; Available from: https://apps.who.int/iris/handle/10665/326178.
6. Deeks, E.D., *Fexinidazole: First global approval.* Drugs, 2019. **79**(2): p. 215-220.
7. Franco, J.R., P.P. Simarro, A. Diarra, and J.G. Jannin, *Epidemiology of human African trypanosomiasis.* Clinical epidemiology, 2014. **6**: p. 257.
8. Lindner, A.K., et al., *New WHO guidelines for treatment of gambiense human African trypanosomiasis including fexinidazole: substantial changes for clinical practice.* Lancet Infect Dis, 2020. **20**(2): p. e38-e46.
9. Steverding, D.J.P. and vectors, *The history of African trypanosomiasis.* 2008. **1**(1): p. 3.
10. Berrang-Ford, L., J. Lundine, and S. Breau, *Conflict and human African trypanosomiasis.* Social Science & Medicine, 2011. **72**(3): p. 398-407.
11. World Health Organization, *Control and surveillance of African trypanosomiasis: report of a WHO expert committee.* 1998.
12. Ceita, C., *Censo 2014. Resultados definitivos do recenseamento geral da população geral e da habitação de Angola 2014.* 2016: p. 213.
13. Simarro, P.P., et al., *Monitoring the progress towards the elimination of gambiense human African trypanosomiasis.* PLoS neglected tropical diseases, 2015. **9**(6): p. e0003785.
14. Truc, P., et al., *Epidemiological aspects of the transmission of the parasites causing human African trypanosomiasis in Angola.* Annals of Tropical Medicine & Parasitology, 2011. **105**(3): p. 261-265.
15. Stanghellini, A., T.J.T.M. Josenando, and I. Health, *The situation of sleeping sickness in Angola: a calamity.* 2001. **6**(5): p. 330-334.
16. Ruiz, J.A., P.P. Simarro, and T.J.B.o.t.W.H.O. Josenando, *Control of human African trypanosomiasis in the Quicama focus, Angola.* 2002. **80**: p. 738-745.
17. Muela, S.H., et al., *Medical syncretism with reference to malaria in a Tanzanian community.* 2002. **55**(3): p. 403-413.
18. World Health Organization, *The promotion and development of traditional medicine: report of a WHO meeting [held in Geneva from 28 November to 2 December 1977].* 1978.
19. World Health Organization. *WHO Traditional Medicine Strategy 2002-2005. World Health Organization. Geneva.* 2002; Available from: http://www.wpro.who.int/health_technology/book_who_traditional_medicine_strategy_2002_2005.pdf
20. Abdullahi, A.A., *Trends and challenges of traditional medicine in Africa.* Afr J Tradit Complement Altern Med, 2011. **8**(5 Suppl): p. 115-23.
21. World Health Organization, *Promoting the Role of Traditional Medicine in Health Systems: A Strategy for the African Region (document AFR/RC50/9).* Regional Office for Africa, Temporary Location, Harare, Zimbabwe, 2001: p. 1-25.
22. World Health Organization, *General guidelines for methodologies on research and evaluation of traditional medicine*. 2000, Geneva: World Health Organization.

23. WHO, *WHO traditional Medicine Strategy: 2014-2023. World Health Organization.* 2013: p. 76.
24. World Health Organization. *WHO Global Report on Tradititional And Complementary Medicine 2019. World Health Organization.* 2019; Available from: https://www.who.int/traditional-complementary-integrative-medicine/WhoGlobalReportOnTraditionalAndComplementaryMedicine2019.pdf?ua=1.
25. Kasilo, O.M.J., C. Wambebe, J.-B. Nikiema, and J.J.B.g.h. Nabyonga-Orem, *Towards universal health coverage: advancing the development and use of traditional medicines in Africa.* 2019. **4**(Suppl 9): p. e001517.
26. Organization, W.H., *Stratégie de l' OMS pour la médecine traditionnelle pour 2002-2005*. 2002, Genève : Organisation mondiale de la Santé.
27. Graz, B., et al., *Screening of traditional herbal medicine: first, do a retrospective study, with correlation between diverse treatments used and reported patient outcome.* 2005. **101**(1-3): p. 338-339.
28. Graz, B., E. Elisabetsky, and J.J.J.o.e. Falquet, *Beyond the myth of expensive clinical study: Assessment of traditional medicines.* 2007. **113**(3): p. 382-386.
29. World Health Organization. *Programme on Traditional Medicine. Guidelines for the assessment of herbal medicnes. WHO.* 1991; Available from: https://apps.who.int/iris/handle/10665/58865.
30. Amuamuta, A., K.J.A.J.o.P. Na-Bangchang, and Pharmacology, *A review of ethnopharmacology of the commonly used antimalarial herbal agents for traditional medicine practice in Ethiopia.* 2015. **9**(25): p. 615-627.
31. Willcox, M.L. and G.J.B. Bodeker, *Traditional herbal medicines for malaria.* 2004. **329**(7475): p. 1156-1159.
32. Graz, B., A.Y. Kitua, and H.M.J.M.j. Malebo, *To what extent can traditional medicine contribute a complementary or alternative solution to malaria control programmes?* 2011. **10**(S1): p. S6.
33. Willcox, M.L.J.J.o.P.H., *A clinical trial of'AM', a Ugandan herbal remedy for malaria.* 1999. **21**(3): p. 318-324.
34. Aracil, A. and J. Green, *Plants with antimalarial properties: A systematic review of the current clinical evidence.* European Journal of Integrative Medicine, 2019. **28**: p. 76-85.
35. Aracil, A. and J. Green, *Plants with antimalarial properties: A systematic review of the current clinical evidence.* European Journal of Integrative Medicine, 2019(28): p. 76-85.
36. Diallo, D., et al., *Knowledge of traditional healers on malaria in Kendi (Bandiagara) and Finkolo (Sikasso) in Mali.* 2007. **22**(4): p. 1-8.
37. Diallo, D., et al., *Malaria treatment in remote areas of Mali: use of modern and traditional medicines, patient outcome.* 2006. **100**(6): p. 515-520.
38. Willcox, M.L., et al., *Argemone mexicana decoction for the treatment of uncomplicated falciparum malaria.* 2007. **101**(12): p. 1190-1198.
39. Graz, B., et al., *Argemone mexicana decoction versus artesunate-amodiaquine for the management of malaria in Mali: policy and public-health implications.* 2010. **104**(1): p. 33-41.
40. Willcox, M., *Improved traditional phytomedicines in current use for the treatment of malaria.* Planta Med, 2011(77): p. 662-671.
41. Bodeker, G. and B. Graz, *Traditional medicine*, in *Hunter's Tropical Medicine and Emerging Infectious Diseases*. 2020, Elsevier. p. 194-199.
42. Haidara, M., et al., *In vivo validation of anti-malarial activity of crude extracts of Terminalia macroptera, a Malian medicinal plant.* 2018. **17**(1): p. 68.

43. Haidara, M., *Contribution à l'étude de l'activité pharmacologique de Terminalia macroptera Guill. et Perr.(Combretaceae) dans le but de l'élaboration d'un médicament traditionnel amélioré au Mali (Afrique de l'Ouest). Thèse doctorale.* 2018.
44. Willcox, M., et al., *Improved traditional medicines in Mali.* 2012. **18**(3): p. 212-220.
45. Bony, N.F., *Stratégie analytique des tradimédicaments: établissement de profils chromatographiques des métabolites phytochimiques apolaires. Thèse de doctorat. Université de Paris-Sud et Université Felix HouphouetBoigny Abidjan-Cocody.* 2013, Paris 11.
46. Diabate, D.M., *Etude de la phytochimie et des activités biologiques d'une recette traditionnelle utilisée dans le traitement de l'hypertrophie bénigne de la prostate au Mali. Thèse de Doctorat. Faculté de Pharmacie. .* 2006.
47. Ibrahim, M.A., A. Mohammed, M.B. Isah, and A.B. Aliyu, *Anti-trypanosomal activity of African medicinal plants: A review update.* Journal of ethnopharmacology, 2014. **154**(1): p. 26-54.
48. Simoben, C.V., F. Ntie-Kang, S.H. Akone, and W. Sippl, *Compounds from African medicinal plants with activities against selected parasitic diseases: Schistosomiasis, trypanosomiasis and leishmaniasis.* Natural products and bioprospecting, 2018. **8**(3): p. 151-169.
49. Ioset, J.-R.J.C.O.C., *Natural products for neglected diseases: a review.* 2008. **12**(8): p. 643-666.
50. Nwodo, N.J., et al., *Anti-trypanosomal activity of Nigerian plants and their constituents.* 2015. **20**(5): p. 7750-7771.
51. Ibrahim, M.A., et al., *Phenolics-rich fraction of Khaya senegalensis stem bark: antitrypanosomal activity and amelioration of some parasite-induced pathological changes.* Pharm Biol, 2013. **51**(7): p. 906-13.
52. Ibrahim, M.A., A. Mohammed, M.B. Isah, and A.B. Aliyu, *Anti-trypanosomal activity of African medicinal plants: a review update.* J Ethnopharmacol, 2014. **154**(1): p. 26-54.
53. Cretton, S., et al., *Antitrypanosomal quinoline alkaloids from the roots of Waltheria indica.* 2014. **77**(10): p. 2304-2311.
54. Zimmermann, S., et al., *Cynaropicrin: the first plant natural product with in vivo activity against Trypanosoma brucei.* 2012. **78**(06): p. 553-556.
55. United Nations. *Convention on Biological Diversity. Rio de Janeiro: Secretariat of the Convention on Biological Diversity*. 1992; Available from: https://www.cbd.int/convention/text/
56. Secretariat of the Convention on Biological Diversity. *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity*. 2011; Available from: https://www.cbd.int/abs/doc/protocol/nagoya-protocol-en.pdf
57. The Federal Assembly of the Swiss Confederation. *Federal Act on the Protection of Nature and Cultural Heritage.* 1996; Available from: https://www.admin.ch/opc/en/classified-compilation/19660144/index.html
58. The Swiss Federal Council. *Ordinance on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilisation*. 2015; Available from: https://www.admin.ch/opc/en/classified-compilation/20150120/index.html#a8.
59. Direction of National Institute for Biodiversity and Protected Areas (INBAC). *Interim National Reports on the implementation of the Nagoya Protocol. Angola. ABSCH-NR-AO-246393-1. 30 May 2019*. 2019; Available from: https://absch.cbd.int/countries/AO.
60. Heinrich, M., et al., *Best practice in research: consensus statement on ethnopharmacological field studies–ConSEFS.* 2018. **211**: p. 329-339.

61. Jorge Lima, A. *Bioética em Angola: iniciativas e desafios. In Bioética nos Países de Língua Oficial Portuguesa -Justiça e Solidariedade. .* in *Conferência de Bioética nos Países de Língua Oficial Portuguesa*. 2014. Lisboa.
62. World Health Organization, *United Nations Declaration on the Rights of Indigenous Peoples. WHO.* 2007.
63. Guideline, I.H.J.C.S., *Integrated addendum to ICH E6 (R1): guideline for good clinical practice E6 (R2).* 2015. **2**: p. 1-60.
64. Biber-Klemm, S. and S.I. Martinez, *Utilization of genetic resources and associated traditional knowledge in academic research. A good practice guide for access and benefit-sharing*. Vol. 11. 2016: Swiss Academies of Arts and Sciences.
65. Angolan National Assembly. *Lei do Património Cultural. Lei n°14/05 de 7 de Outubro de 2005. WIPO Lex No. AO018*. 2005; Available from: https://wipolex.wipo.int/en/legislation/details/11009
66. United Nations Educational Scientifc and Cultural Organization. *Convention on the protection and promotion of the diversity of cultural expressions. Paris, 20 October 2005. UNESCO*. 2005; Available from: https://wipolex.wipo.int/en/text/209332.
67. International Society of Ethnobiology. *ISE Code of Ethics (with 2008 additions).* 2006; Available from: http://ethnobiology.net/code-of-ethics/.
68. Amari, A., B. Kablan, and J. Pabst, *Mondialisation et protection des droits de propriété intellectuelle des détenteurs de savoirs en matière de pharmacopée traditionnelle africaine*. 2008, Ethnopharmacologia.
69. Conseil des Ministres Maliens. *Décret N°95-009/P-RM instituant un visa des produits pharmaceutiques.* . Bamako, le 11 janvier 1995; Available from: https://wipolex.wipo.int/en/legislation/details/7509.
70. Wendland, W. and F. Jiao, *Intellectual Property Rights and Traditional Medical Knowledge in Africa*, in *African Indigenous Medical Knowledge and Human Health*

2018, CRC Press. p. p.247.

71. Worl Intellectual Property Organization, *Intellectual Property Needs and Expectations of Traditional Knowledge Holders: WIPO Report on Fact-finding Missions on Intellectual Property and Traditional Knowledge (1998-1999).* 2001: WIPO.
72. Worl Intellectual Property Organization, *WIPO intellectual property handbook: Policy, law and use.* . Vol. 489. 2004: WIPO.
73. YENTCHARE, P.-y. and S. Lavallée, *Protocole De Nagoya: Les Multiples Facettes De Sa Mise En Oeuvre*, in *Liaison Energie-francophonie*. 2016. p. 120.
74. WHO. *Fact sheets: Trypanosomiasis, human African (sleeping sickness)*. 2019 2019, April 2019 2019, July 7]; Available from: http://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness.
75. Mesu, V., et al., *Oral fexinidazole for late-stage African Trypanosoma brucei gambiense trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial.* Lancet, 2018. **391**(10116): p. 144-154.
76. Baker, C.H. and S.C. Welburn, *The long wait for a new drug for human African trypanosomiasis.* Trends in parasitology, 2018. **34**(10): p. 818-827.
77. Gurib-Fakim, A. and M.F. Mahomoodally, *African Flora as Potential Sources of Medicinal Plants: Towards the Chemotherapy of Major Parasitic and Other Infectious Diseases: A Review.* Jordan Journal of Biological Sciences, 2013. **147**(624): p. 1-8.
78. J Schmidt, T., et al., *The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases-part II.* Current medicinal chemistry, 2012. **19**(14): p. 2176-2228.

79. Ogungbe, I. and W. Setzer, *The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases—Part III: In-silico molecular docking investigations.* Molecules, 2016. **21**(10): p. 1389.
80. Mahomoodally, M.F., *Traditional medicines in Africa: an appraisal of ten potent African medicinal plants.* Evidence-Based Complementary and Alternative Medicine, 2013. **2013**.
81. Nwodo, N., et al., *Anti-trypanosomal activity of Nigerian plants and their constituents.* Molecules, 2015. **20**(5): p. 7750-7771.
82. Gehrig, S. and T. Efferth, *Development of drug resistance in Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense. Treatment of human African trypanosomiasis with natural products.* International journal of molecular medicine, 2008. **22**(4): p. 411-419.
83. Mwangi, V.I., R.M. Mumo, A. Nyachieo, and N. Onkoba, *Herbal medicine in the treatment of poverty associated parasitic diseases: A case of sub-Saharan Africa.* Journal of herbal medicine, 2017. **10**: p. 1-7.
84. Bossard, E., *La médecine traditionnelle au centre et à l'ouest de l'Angola.* 1996.
85. Urso, V., M.A. Signorini, M. Tonini, and P. Bruschi, *Wild medicinal and food plants used by communities living in mopane woodlands of southern Angola: results of an ethnobotanical field investigation.* Journal of ethnopharmacology, 2016. **177**: p. 126-139.
86. Leyens, T. and W. Lobin, *Manual de plantas úteis de Angola*. 2009: Bischöfliches Hilfswerk Misereor.
87. Göhre, A., et al., *Plants from disturbed savannah vegetation and their usage by Bakongo tribes in Uíge, Northern Angola.* Journal of ethnobiology and ethnomedicine, 2016. **12**(1): p. 42.
88. Lautenschläger, T., *Riquezas naturais de Uíge: uma breve introdução sobre o estado atual, a utilização, a ameaça ea preservação da biodiversidade*. 2014: Techn. Univ.
89. Heinze, C., et al., *First ethnobotanical analysis of useful plants in Cuanza Norte, North Angola.* Res Rev J Bot Sci, 2017. **6**: p. 44-53.
90. Lautenschläger, T., et al., *First large-scale ethnobotanical survey in the province of Uíge, northern Angola.* Journal of ethnobiology and ethnomedicine, 2018. **14**(1): p. 51.
91. Videira, C., J.M. Pedro, and S.V. Nery, *Rastreio etnobotânico nas comunas de Caxito, Mabubas e Úcua (Província do Bengo): resultados preliminares.* 2011.
92. Van Dúnem, M., *Plantas medicinais de Angola: medicamentos ao alcance de todos.* Cooperação Portuguesa/ Embaixada de Portugal, Luanda, 1994(1st ed.): p. 78.
93. Da Costa, E., Pedro, M., *Plantas Medicinais de Angola.* Universidade Agostinho Neto, Centro de Botânica, 2013.
94. Pompermaier, L., et al., *Medicinal plants of northern Angola and their anti-inflammatory properties.* Journal of ethnopharmacology, 2018. **216**: p. 26-36.
95. WHO. *Global Health Observatory data repository, Number of new reported cases (T.b. gambiense).Data by country*. 2016 2019, July 7]; Available from: http://apps.who.int/gho/data/node.main.A1636?lang=en.
96. Gossweiler, J., *Nomes indigenas de plantas de Angola ed do Angola*. 1953, Imprensa Nacional do Angola, Luanda, Angola). Portugese.
97. Figueiredo, E., *Plants of Angola/Plantas de Angola.* 2008.
98. Lardos, A. and M. Heinrich, *Continuity and change in medicinal plant use: the example of monasteries on Cyprus and historical iatrosophia texts.* Journal of ethnopharmacology, 2013. **150**(1): p. 202-214.
99. Diop, E.A., et al., *Survey on medicinal plants traditionally used in Senegal for the treatment of tuberculosis (TB) and assessment of their antimycobacterial activity.* Journal of ethnopharmacology, 2018. **216**: p. 71-78.

100. WHO, *National policy on traditional medicine and regulation of herbal medicines: Report of a WHO global survey. World Health Organization.* 2005.
101. Santos, P.A.D.d., *Recurso à medicina tradicional no tratamento de crianças angolanas: estudo exploratório no Hospital Pediátrico do Lubango*. 2012.
102. Ondicho, J., J. Ochora, E. Matu, and J. Mutai. *Factors associated with use of herbal medicine among patients in herbal clinics in Gucha district, Kenya*. in *Scientific Conference Proceedings*. 2016.
103. Fakeye, T.O., R. Adisa, and I.E. Musa, *Attitude and use of herbal medicines among pregnant women in Nigeria.* BMC Complementary and alternative medicine, 2009. **9**(1): p. 53.
104. Mpanya, A., et al., *Should I get screened for sleeping sickness? A qualitative study in Kasai province, Democratic Republic of Congo.* PLoS neglected tropical diseases, 2012. **6**(1): p. e1467.
105. Stekelenburg, J., et al., *Health care seeking behaviour and utilisation of traditional healers in Kalabo, Zambia.* Health policy, 2005. **71**(1): p. 67-81.
106. Simarro, P.P., et al., *Mapping the capacities of fixed health facilities to cover people at risk of gambiense human African trypanosomiasis.* International journal of health geographics, 2014. **13**(1): p. 4.
107. Kuikumbi, F.M., *Bulletin d'information N°19, Mai 2018. Plate forme régionale de recherche clinique Trypanosomiase Humaine Africaine THA.* 2018: p. 40.
108. Luis, S., et al., *Health-seeking norms for tuberculosis symptoms in southern Angola: implications for behaviour change communications.* The International Journal of Tuberculosis and Lung Disease, 2011. **15**(7): p. 943-948.
109. Audet, C.M., et al., *HIV knowledge and health-seeking behavior in Zambézia Province, Mozambique.* SAHARA-J: Journal of Social Aspects of HIV/AIDS, 2012. **9**(1): p. 41-46.
110. Naidoo, P., *Other health-seeking behaviour of HIV and AIDS patients visiting private sector doctors in the eThekwini Metropolitan Municipality of KwaZulu-Natal.* South African Family Practice, 2014. **56**(4): p. 223-228.
111. Hatchett, L., et al., *Health-seeking patterns for AIDS in Malawi.* AIDS care, 2004. **16**(7): p. 827-833.
112. SaMal, J., *Health seeking behaviour among tuberculosis patients in India: a systematic review.* Journal of clinical and diagnostic research: JCDR, 2016. **10**(10): p. LE01.
113. Sodi, T. and T. Phethi, *Influences on help-seeking pathways among people with chronic illness and disease in a rural South African community.* Journal of Psychology in Africa, 2017. **27**(3): p. 286-289.
114. Jørstad, M.D., et al., *Diagnostic delay in extrapulmonary tuberculosis and impact on patient morbidity: A study from Zanzibar.* PloS one, 2018. **13**(9): p. e0203593.
115. Ngwira, L.-G., et al., *Delay in seeking care for tuberculosis symptoms among adults newly diagnosed with HIV in rural Malawi.* The international journal of tuberculosis and lung disease, 2018. **22**(3): p. 280-286.
116. Santos, E., Ó. Felgueiras, O. Oliveira, and R. Duarte, *Diagnosis delay of tuberculosis in the Huambo province, Angola.* Pulmonology, 2018. **24**(5): p. 294-299.
117. Odiit, M., et al., *Assessing the patterns of health-seeking behaviour and awareness among sleeping-sickness patients in eastern Uganda.* Annals of Tropical Medicine & Parasitology, 2004. **98**(4): p. 339-348.
118. Hasker, E., et al., *Health care-seeking behaviour and diagnostic delays for Human African Trypanosomiasis in the Democratic Republic of the Congo.* Tropical Medicine & International Health, 2011. **16**(7): p. 869-874.
119. Mpanya, A., et al., *From health advice to taboo: community perspectives on the treatment of sleeping sickness in the Democratic Republic of Congo, a qualitative study.* PLoS neglected tropical diseases, 2015. **9**(4): p. e0003686.

120. Robays, J., et al., *Drug toxicity and cost as barriers to community participation in HAT control in the Democratic Republic of Congo.* Tropical Medicine & International Health, 2007. **12**(2): p. 290-298.
121. Costa, E., A. Dombo, and M. Paula, *Plantas Ameaçadas em Angola*. 2009.
122. Pousset, J.-L., *Plantes médicinales d'Afrique: Comment les reconnaitre et les utiliser: Ouvrage publié avec le soutien du Conseil général des Bouches-du-Rhône*. 2004: Secum/Edisud.
123. Maiga, A., K.E. Malterud, D. Diallo, and B.S. Paulsen, *Antioxidant and 15-lipoxygenase inhibitory activities of the Malian medicinal plants Diospyros abyssinica (Hiern) F. White (Ebenaceae), Lannea velutina A. Rich (Anacardiaceae) and Crossopteryx febrifuga (Afzel) Benth.(Rubiaceae).* Journal of ethnopharmacology, 2006. **104**(1-2): p. 132-137.
124. Banzouzi, J., et al., *Survey of analgesic plants used by tradipractitioners in Congo Brazzaville.* 2008. **4**(2): p. 176-185.
125. Staner, P. and R. Boutique, *Matériaux pour l'étude des plantes médicinales indigènes du Congo Belge. Mém. Institut royal colonial belge, Section des Sc. naturelles et médicales, Collection in-8°, fasc. 6 et dernier, 228 p.*, in *Référence HS 06*. 1937: Base de données Prélude.
126. Salawu, O., B. Chindo, A. Tijani, and B. Adzu, *Analgesic, anti-inflammatory, antipyretic and antiplasmodial effects of the methanolic extract of Crossopteryx febrifuga.* J Med Plant Res, 2008. **2**(8): p. 213-218.
127. Tomás-Barberán, F.A. and K. Hostettmann, *A cytotoxic triterpenoid and flavonoids from Crossopteryx febrifuga.* Planta medica, 1988. **54**(03): p. 266-267.
128. Tasdemir, D., et al., *Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies.* Antimicrobial agents and chemotherapy, 2006. **50**(4): p. 1352-1364.
129. Kharb, M., S. Beniwal, and V. Kumar, *HERBAL DRUGS USED FOR THE TREATMENT OF DIABETES: A REVIEW.* International Journal of Pharmaceutical Research and Medicinal Plants, 2019. **2**(1): p. 01-18.
130. Leung, L., et al., *Anti-diabetic and hypoglycaemic effects of Momordica charantia (bitter melon): a mini review.* British Journal of Nutrition, 2009. **102**(12): p. 1703-1708.
131. Ahmad, N., et al., *Momordica charantia: for traditional uses and pharmacological actions.* Journal of Drug Delivery and Therapeutics, 2016. **6**(2): p. 40-44.
132. Beloin, N., et al., *Ethnomedicinal uses of Momordicacharantia (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity.* Journal of Ethnopharmacology, 2005. **96**(1-2): p. 49-55.
133. Mokganya, M. and M. Tshisikhawe, *Medicinal uses of selected wild edible vegetables consumed by Vhavenda of the Vhembe District Municipality, South Africa.* South African Journal of Botany, 2019. **122**: p. 184-188.
134. Konkon, N., D. Ouatara, W. Kpan, and T. Kouakou, *Medicinal plants used for treatment of diabetes by traditional practitioners in the markets of Abidjan district in Côte d'Ivoire.* J. Med. Plants Stud, 2017. **5**: p. 39-48.
135. Ondo, J.P., et al., *In vitro antiplasmodial activity and cytotoxicity of extracts and fractions of Vitex madiensis, medicinal plant of Gabon.* Tropical Medicine & International Health, 2012. **17**(3): p. 316-321.
136. Diarra, N., et al., *Ethnobotanical study of plants used against malaria in Sélingué subdistrict, Mali.* Journal of ethnopharmacology, 2015. **166**: p. 352-360.
137. Nacoulma-Ouédraogo, O., *Plantes médicinales et pratiques traditionnelles au Burkina: cas du plateau central*. 1996, Thèse de Doctorat Es Sciences Naturelles, Université de Ouagadougou.

138. Nwodo, N., et al., *Evaluation of the in vitro trypanocidal activity of methylated flavonoid constituents of Vitex simplicifolia leaves.* BMC complementary and alternative medicine, 2015. **15**(1): p. 82.
139. Sifuma, S.W., *Vitex doniana sweet Verbanaceae*. 2011, DEPARTMENT OF PHARMACOLOGY AND PHARMACOGNOSY, SCHOOL OF PHARMACY COLLEGE OF ….
140. Mbuta, K.k., et al., *Plantes médicinales de traditions. Province de l'Equateur- R.D. Congo*. 2012, Kinshasa: Institut de Recherche en Science de la Santé (I.R.S.S.). 420.
141. Nwodo, N., et al., *Trypanocidal activity of flavonoids from Vitex simplicifolia.* Planta Medica, 2013. **79**(13): p. SL3.
142. Atawodi, S., et al., *Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria.* Journal of Ethnopharmacology, 2002. **79**(2): p. 279-282.
143. Shilema, A., K. Zerom, and A. Mussa, *Ethnoveterinary practices against animal trypanosomosis in Amaro district, Southern Ethiopia.* Int J Med Plants Res, 2013. **2**: p. 238-41.
144. Adjanohoun, E.J. and P. Sita, *Contribution aux études ethnobotaniques et floristiques en République populaire du Congo: rapport présenté à l'A.C.C.T*. Vol. 1 vol. . 1988: Agence de Coopération Culturelle et Technique. Paris. 605.
145. Bouquet, A. and M. Debray, *Plantes medicinales de la Côte d'Ivoire.: Travaux et Documents de L'ORSTOM No. 32, Paris. 1974.* . 1974, Pergamon. p. 231.
146. Bizimana, N., et al., *Evaluation of medicinal plants from Mali for their in vitro and in vivo trypanocidal activity.* Journal of ethnopharmacology, 2006. **103**(3): p. 350-356.
147. Hamill, F., et al., *Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays.* Journal of Ethnopharmacology, 2003. **84**(1): p. 57-78.
148. Freiburghaus, F., et al., *In vitro antitrypanosomal activity of African plants used in traditional medicine in Uganda to treat sleeping sickness.* Tropical Medicine & International Health, 1996. **1**(6): p. 765-771.
149. Kerharo, J. and J.G. Adam, *Les plantes médicinales, toxiques et magiques des Niominka et des Socé des Iles du Saloum (Sénégal).* Acta Trop Suppl, 1964. **8**: p. 279-334.
150. Diafouka, A., *Analysis of medicinal uses in four regions of Congo-Brazzaville. Doctoral thesis.*, in *Faculté des Sciences, Laboratoire de Botanique Systématique et de Phytosociologie* 1997, Université Libre de Bruxelles. p. 431.
151. Maikai, V., U. Abubakar, A. Salman, and T. Inuwa, *Preliminary Survey of Medicinal Plants Used in Treatment of Animal Trypanosomosis in Kaduna State, Nigeria.* Ethnobotanical Leaflets, 2010. **2010**(3): p. 9.
152. Mongalo, N.I., L. McGaw, J. Finnie, and J. Van Staden, *Securidaca longipedunculata Fresen (Polygalaceae): A review of its ethnomedicinal uses, phytochemistry, pharmacological properties and toxicology.* Journal of ethnopharmacology, 2015. **165**: p. 215-226.
153. Moukouta, S. and E. Pewzner-Apeloig. *Thérapies traditionnelles-thérapies modernes en milieu psychiatrique au Congo. Syncrétisme ou interférence?* in *Annales Médico-psychologiques, revue psychiatrique*. 2002. Elsevier.
154. Sobiecki, J., *A review of plants used in divination in southern Africa and their psychoactive effects.* Southern African Humanities, 2008. **20**(2): p. 333-351.
155. Ioset, J.-R., *Natural products for neglected diseases: a review.* Current Organic Chemistry, 2008. **12**(8): p. 643-666.
156. Nwodo, N.J., et al., *Anti-Trypanosomal Activity of Nigerian Plants and Their Constituents.* Molecules, 2015. **20**(5): p. 7750-7771.
157. Ueda-Nakamura, T., et al., *Antileishmanial activity of Eugenol-rich essential oil from Ocimum gratissimum.* Parasitology international, 2006. **55**(2): p. 99-105.

158. Adamu, M., C. Nwosu, and R. Agbede, *Anti-trypanosomal effects of aqueous extract of Ocimum gratissimum (Lamiaceae) leaf in rats infected with Trypanosoma brucei brucei.* African Journal of Traditional, Complementary and Alternative Medicines, 2009. **6**(3).
159. Muganza, D.M., et al., *In vitro antiprotozoal and cytotoxic activity of 33 ethonopharmacologically selected medicinal plants from Democratic Republic of Congo.* Journal of ethnopharmacology, 2012. **141**(1): p. 301-308.
160. Olukunle, J., et al., *In vivo antitrypanosomal evaluation of some medicinal plant extracts from Ogun State, Nigeria.* Science World Journal, 2010. **5**(1).
161. Kpoviessi, B.G.K., et al., *In vitro antitrypanosomal and antiplasmodial activities of crude extracts and essential oils of Ocimum gratissimum Linn from Benin and influence of vegetative stage.* Journal of ethnopharmacology, 2014. **155**(3): p. 1417-1423.
162. Zeenat, F., M.S. Ravish, W. Ahmad, and I. Ahmad, *Therapeutic, phytochemistry and pharmacology of Azadirachta indica: A review.* International Journal of Unani and Integrative Medicine, 2018. **2**(1): p. 20-28.
163. Saleem, S., G. Muhammad, M.A. Hussain, and S.N.A. Bukhari, *A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of Azadirachta indica.* Phytotherapy research, 2018. **32**(7): p. 1241-1272.
164. Khetarpal, S., *Neem: A Wonder Tree.* Asia-Paci ic Biotech News, 2010. **14**(18): p. 18-20.
165. Subapriya, R. and S. Nagini, *Medicinal properties of neem leaves: a review.* Current Medicinal Chemistry-Anti-Cancer Agents, 2005. **5**(2): p. 149-156.
166. Sofowora, A., *Research on medicinal plants and traditional medicine in Africa.* The Journal of Alternative and Complementary Medicine, 1996. **2**(3): p. 365-372.
167. Tepongning, R.N., et al., *Hydroethanolic Extracts of Erigeron floribundus and Azadirachta indica Reduced Plasmodium berghei Parasitemia in Balb/c Mice.* Evidence-Based Complementary and Alternative Medicine, 2018. **2018**.
168. MacKinnon, S., et al., *Antimalarial activity of tropical Meliaceae extracts and gedunin derivatives.* Journal of natural products, 1997. **60**(4): p. 336-341.
169. Priyanka, J., L. Hingorani, and K. Nilima, *Pharmacodynamic evaluation for antiplasmodial activity of Holarrhena antidysentrica (Kutaja) and Azadirachta indica (Neemb) in Plasmodium berghei infected mice model.* Asian Pacific journal of tropical medicine, 2013. **6**(7): p. 520-524.
170. Rochanakij, S., Y. Thebtaranonth, C. Yenjai, and Y. Yuthavong, *Nimbolide, a constituent of Azadirachta indica, inhibits Plasmodium falciparum in culture.* The Southeast Asian journal of tropical medicine and public health, 1985. **16**(1): p. 66-72.
171. NOK, A.J., et al., *Trypanocidal potentials of Azadirachta indica: In vivo activity of leaf extract against Trypanosoma brucei brucei.* Journal of clinical biochemistry and nutrition, 1993. **15**(2): p. 113-118.
172. Mbaya, A.W., U.I. Ibrahim, O.T. God, and S. Ladi, *Toxicity and potential anti-trypanosomal activity of ethanolic extract of Azadirachta indica (Maliacea) stem bark: an in vivo and in vitro approach using Trypanosoma brucei.* Journal of ethnopharmacology, 2010. **128**(2): p. 495-500.
173. Seyoum, A., et al., *Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against Anopheles gambiae: ethnobotanical studies and application by thermal expulsion and direct burning.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 2002. **96**(3): p. 225-231.
174. Kweka, E.J., et al., *Longitudinal evaluation of Ocimum and other plants effects on the feeding behavioral response of mosquitoes (Diptera: Culicidae) in the field in Tanzania.* Parasites & vectors, 2008. **1**(1): p. 42.

175. Kiplang'at, K.P. and R. Mwangi, *Repellent activities of Ocimum basilicum, Azadirachta indica and Eucalyptus citriodora extracts on rabbit skin against Aedes aegypti.* Journal of Entomology and Zoology Studies, 2013. **1**(5): p. 84-91.
176. Abiy, E., T. Gebre-Michael, M. Balkew, and G. Medhin, *Repellent efficacy of DEET, MyggA, neem (Azedirachta indica) oil and chinaberry (Melia azedarach) oil against Anopheles arabiensis, the principal malaria vector in Ethiopia.* Malaria journal, 2015. **14**(1): p. 187.
177. Kayode, J., L. Aleshinloye, and O. Ige, *Ethnomedicinal use of plant species in Ijesa Land of Osun State, Nigeria.* Ethnobotanical leaflets, 2008. **2008**(1): p. 20.
178. John-Africa, L., M.S. Idris-Usman, B. Adzu, and K.S. Gamaniel, *Protective effects of the aqueous extract of Nymphaea lotus L.(Nymphaeaceae) against ethanol-induced gastric ulcers.* International Journal of Biological and Chemical Sciences, 2012. **6**(5): p. 1917-1925.
179. Randrianarivelojosia, M., et al., *Plants traditionally prescribed to treat tazo (malaria) in the eastern region of Madagascar.* Malaria Journal, 2003. **2**(1): p. 25.
180. Garba, M.H., et al., *In vivo trypanocidal activity of Nymphaea lotus Linn. methanol extract against Trypanosoma brucei brucei.* Asian Pacific Journal of Tropical Disease, 2015. **5**(10): p. 808-812.
181. Abubakar, A., et al., *Antitrypanosomal and haematological effects of selected Nigerian medicinal plants in Wistar rats.* Biokemistri, 2005. **17**(2): p. 95-99.
182. Haruna, Y., et al., *In vivo trypanocidal effect of aqueous root extracts of Securidaca longepedunculata and its phytochemical analysis.* African Journal of Pharmacy and Pharmacology, 2013. **7**: p. 2838-2842.
183. Freiburghaus, F., R. Kaminsky, M. Nkunya, and R. Brun, *Evaluation of African medicinal plants for their in vitro trypanocidal activity.* Journal of ethnopharmacology, 1996. **55**(1): p. 1-11.
184. Freiburghaus, F., A. Steck, H. Pfander, and R. Brun, *Bioassay-guided isolation of a diastereoisomer of kolavenol from Entada abyssinica active on Trypanosoma brucei rhodesiense.* Journal of ethnopharmacology, 1998. **61**(3): p. 179-183.
185. Mesia, G., et al., *Antiprotozoal and cytotoxic screening of 45 plant extracts from Democratic Republic of Congo.* Journal of ethnopharmacology, 2008. **115**(3): p. 409-415.
186. Atindehou, K.K., et al., *Antitrypanosomal and antiplasmodial activity of medicinal plants from Côte d'Ivoire.* Journal of Ethnopharmacology, 2004. **90**(2-3): p. 221-227.
187. Nyasse, B., et al., *Inhibition of both Trypanosoma brucei bloodstream form and related glycolytic enzymes by a new kolavic acid derivative isolated from Entada abyssinica.* Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2004. **59**(11): p. 873-875.
188. Nibret, E., M.L. Ashour, C.D. Rubanza, and M. Wink, *Screening of some Tanzanian medicinal plants for their trypanocidal and cytotoxic activities.* Phytotherapy Research, 2010. **24**(6): p. 945-947.
189. Sempombe, J., et al., *Preliminary in vivo antitrypanosomal activity and cytotoxicity of Entada abyssinica, Securinega virosa and Ehretia amoena.* East and Central African Journal of Pharmaceutical Sciences, 2014. **17**(2): p. 37-43.
190. Madubunyi, I., *Antihepatotoxic and trypanocidal activities of the ethanolic extract of Nauclea latifolia root bark.* Journal of herbs, spices & medicinal plants, 1996. **3**(2): p. 23-35.
191. Youan, B., et al., *In vivo evaluation of sixteen plant extracts on mice inoculated with Trypanosoma brucei gambiense.* Bulletin of the World Health Organization, 1997. **75**(4): p. 343.
192. Camacho, M., et al., *Screening of plant extracts for antiprotozoal and cytotoxic activities.* Journal of ethnopharmacology, 2003. **89**(2-3): p. 185-191.

193. Maikai, V. and P. Kobo, *Preliminary studies on the in vitro antitrypanosomal activity of aqueous and methanolic crude extracts of stem bark of Nauclea latifolia on Trypanosoma congolense.* Journal of Medicinal Plants Research, 2008. **2**(6): p. 115-118.
194. Igoli, J.O., A.I. Gray, C.J. Clements, and H.A. Mouad, *Anti-Trypanosomal Activity and cytotoxicity of some compounds and extracts from Nigerian Medicinal Plants*, in *Phytochemicals-Bioactivities and impact on Health*. 2011, IntechOpen.
195. Olanrewaju, C.A., H.S. Idris, and S.K. Okwute, *Investigation on the trypanocidal effects of aqueous extracts of Vernonia amygdalina and Nauclea latifolia in albino rats.* Researcher, 2014. **6**: p. 61-69.
196. Longdet, I., H. Achemu, and C. Okanlawon, *Potentials of Methanolic Extract of N. Latifolia Stem Bark Against T. Congolense Infection in Experimental Rats.* 2014.
197. Yusuf, A.B., et al., *Preliminary evaluation for anti-trypanosomal activity of aqueous stem bark extract of Crossopteryx febrifuga in Trypanosoma congolense-infected rats.* Journal of Pharmacy & Bioresources, 2005. **2**(2): p. 111-115.
198. Jiménez-Coello, M., et al., *Assessment of the anti-protozoal activity of crude Carica papaya seed extract against Trypanosoma cruzi.* Molecules, 2013. **18**(10): p. 12621-12632.
199. Julianti, T., et al., *HPLC-based activity profiling for antiplasmodial compounds in the traditional Indonesian medicinal plant Carica papaya L.* Journal of ethnopharmacology, 2014. **155**(1): p. 426-434.
200. Nwodo, N., M. Agbo, and R. Brun, *In vitro and in vivo Antitrypanosomal studies of the leaf extract of Vitex simplicifolia.* Afr. J. Pharm. Res. Dev, 2012. **4**: p. 35-40.
201. Santos, K.K., et al., *Trypanocide, cytotoxic, and antifungal activities of Momordica charantia.* Pharmaceutical biology, 2012. **50**(2): p. 162-166.
202. Phillips, E.A., D.W. Sexton, and D. Steverding, *Bitter melon extract inhibits proliferation of Trypanosoma brucei bloodstream forms in vitro.* Experimental parasitology, 2013. **133**(3): p. 353-356.
203. Atawodi, S., et al., *In vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants.* African Journal of Biotechnology, 2003. **2**(9): p. 317-321.
204. Aderbauer, B., P.-H. Clausen, O. Kershaw, and M.F. Melzig, *In vitro and in vivo trypanocidal effect of lipophilic extracts of medicinal plants from Mali and Burkina Faso.* Journal of ethnopharmacology, 2008. **119**(2): p. 225-231.
205. Haruna, Y., et al., *Bioassay-Guided Fractionation and Anti-Trypanocidal Effect of Fractions and Crude Methanol Roots Extracts of Securidaca Longepedunculata in Mice and Rats.* International Journal of Modern Biochemistry, 2013. **2**(1): p. 1-14.
206. Gabriel, E.I., et al., *Evaluation of Methanol Root Extract of Securidaca longipedunculata for Antitrypanosomal Activity in vitro and in vivo.* Thai Journal of Pharmaceutical Sciences, 2015. **39**(4).
207. Tauheed, A.M., M.M. Suleiman, M. Mamman, and I.A. Lawal, *Ex vivo trypanostatic effect of stem-bark extracts of Securidaca longipedunculata (Fres. Holl) against Trypanosoma brucei brucei.* African Journal of Biotechnology, 2016. **15**(51): p. 2789-2794.
208. Tauheed, A., M. Suleiman, M. Mamman, and A. Lawal, *In vivo antitrypanosomal effects of stem-bark extracts of Securidaca longipedunculata in rats experimentally infected with Trypanosoma brucei brucei.* Sokoto Journal of Veterinary Sciences, 2017. **15**(3): p. 78-84.
209. Ibrahim, M., et al., *Senna occidentalis leaf extract possesses antitrypanosomal activity and ameliorates the trypanosome-induced anemia and organ damage.* Pharmacognosy research, 2010. **2**(3): p. 175.
210. Mustapha, L., O. Angela, and M.Y. David, *Antitrypanosoma activity of the ethanolic leaf extract of Senna occidentalis (Fabaceae) on Trypanosoma brucei brucei infected mice.* International Journal of Basic and Applied Sciences, 2013. **2**(1): p. 32-37.

211. Okpekon, T., et al., *Antiparasitic activities of medicinal plants used in Ivory Coast.* Journal of ethnopharmacology, 2004. **90**(1): p. 91-97.
212. Holetz, F.B., et al., *Effect of essential oil of Ocimum gratissimum on the trypanosomatid Herpetomonas samuelpessoai.* Acta Protozoologica, 2003. **42**(4): p. 269-276.
213. Abiodun, O.O., et al., *Antitrypanosomal activity of some medicinal plants from Nigerian ethnomedicine.* Parasitology research, 2012. **110**(2): p. 521-526.
214. Borges, A.R., et al., *Trypanocidal and cytotoxic activities of essential oils from medicinal plants of Northeast of Brazil.* Experimental Parasitology, 2012. **132**(2): p. 123-128.
215. Adelodun, V.O., et al., *Evaluation of antitrypanosomal and anti inflammatory activities of selected Nigerian medicinal plants in mice.* African Journal of Traditional, Complementary and Alternative Medicines, 2013. **10**(6): p. 469-476.
216. Soh, D., et al., *Xylopioxyde and other bioactive kaurane-diterpenes from Xylopia aethiopica Dunal (Annonaceae).* Journal of Applied Pharmaceutical Science, 2013. **3**(12): p. 013-019.
217. Norhayati, I., et al., *In vitro antitrypanosomal activity of Malaysian plants.* Journal of Tropical Forest Science, 2013: p. 52-59.
218. Alhaji, U.I., et al., *In vitro antitrypanosomal activity, antioxidant property and phytochemical constituents of aqueous extracts of nine Nigerian medicinal plants.* Asian Pacific Journal of Tropical Disease, 2014. **4**(5): p. 348-355.
219. Gachet, M.S., et al., *Assessment of anti-protozoal activity of plants traditionally used in Ecuador in the treatment of leishmaniasis.* Journal of ethnopharmacology, 2010. **128**(1): p. 184-197.
220. Yanes, A., H. Finol, and M. Hasegawa, *extracts from leaves on Typanosoma cruzi growth and.* J. Submicrosc. Cytol. Pathol, 2004. **36**(2): p. 149-154.
221. Ngure, R.M., et al., *Anti-trypanosomal effects of Azadiracta indica (neem) extract on Trypanosoma brucei rhodesiense-infected mice.* 2009.
222. Peter, O., et al., *Evaluation of in vivo antitrypanosomal activity of selected medicinal plant extracts.* Journal of Medicinal Plants Research, 2009. **3**(11): p. 849-854.
223. Githua, M., et al., *New antitrypanosomal tetranotriterpenoids from Azadirachta indica.* African Journal of Traditional, Complementary and Alternative Medicines, 2010. **7**(3).
224. Habila, N., N.C. Humphrey, and A.S. Abel, *Trypanocidal potentials of Azadirachta indica seeds against Trypanosoma evansi.* Veterinary parasitology, 2011. **180**(3-4): p. 173-178.
225. Kamte, S.L.N., et al., *Trypanosoma brucei inhibition by essential oils from medicinal and aromatic plants traditionally used in Cameroon (Azadirachta indica, Aframomum melegueta, Aframomum daniellii, Clausena anisata, Dichrostachys cinerea and Echinops giganteus).* International journal of environmental research and public health, 2017. **14**(7): p. 737.
226. Eddleston, M., et al., *Acute yellow oleander (Thevetia peruviana) poisoning: cardiac arrhythmias, electrolyte disturbances, and serum cardiac glycoside concentrations on presentation to hospital.* Heart, 2000. **83**(3): p. 301-306.
227. Michl, J., M.J. Ingrouille, M.S. Simmonds, and M. Heinrich, *Naturally occurring aristolochic acid analogues and their toxicities.* Natural product reports, 2014. **31**(5): p. 676-693.
228. Liwa, C. and H. Jaka, *Renal Diseases and Use of Medicinal Herbal Extracts: A Concise Update of Reported Literature in Africa.* J Nephrol Renal Ther, 2016. **2**(008).
229. Neuwinger, H.D., *African ethnobotany: poisons and drugs: chemistry, pharmacology, toxicology*. 1996: CRC Press.
230. WHO, *WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems.World Health Organization.* 2004.
231. Simarro, P.P., et al., *Estimating and mapping the population at risk of sleeping sickness.* PLoS neglected tropical diseases, 2012. **6**(10): p. e1859.

232. Kennedy, P.G., *Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness).* The Lancet Neurology, 2013. **12**(2): p. 186-194.
233. Bernhard, S., M. Kaiser, C. Burri, and P. Maser, *Fexinidazole for Human African Trypanosomiasis, the Fruit of a Successful Public-Private Partnership.* Diseases, 2022. **10**(4).
234. Mordt, O.V., A. Tarral, and N. Strub-Wourgaft, *Development and introduction of fexinidazole into the global human African trypanosomiasis program.* The American Journal of Tropical Medicine and Hygiene, 2022. **106**(5 Suppl): p. 61.
235. Vahekeni, N., et al., *Use of herbal remedies in the management of sleeping sickness in four northern provinces of Angola.* 2020. **256**: p. 112382.
236. Tullius Scotti, M., et al., *Natural products as a source for antileishmanial and antitrypanosomal agents.* Combinatorial chemistry & high throughput screening, 2016. **19**(7): p. 537-553.
237. Schmidt, T.J., et al., *The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases - part II.* Curr Med Chem, 2012. **19**(14): p. 2176-228.
238. Oguntoye, S.O., et al., *Evaluation of Selected Nigerian Medicinal Plants for in vitro Antiprotozoal Activity.* The Natural Products Journal, 2018. **8**(3): p. 175-184.
239. Challal, S., et al., *Rational and efficient preparative isolation of natural products by MPLC-UV-ELSD based on HPLC to MPLC gradient transfer.* Planta medica, 2015. **81**(17): p. 1636-1643.
240. Kamatham, S., N. Kumar, and P. Gudipalli, *Isolation and characterization of gallic acid and methyl gallate from the seed coats of Givotia rottleriformis Griff. and their anti-proliferative effect on human epidermoid carcinoma A431 cells.* Toxicology Reports, 2015. **2**: p. 520-529.
241. Cammann, J., K. Denzel, G. Schilling, and G.G. Gross, *Biosynthesis of gallotannins: β-glucogallin-dependent formation of 1, 2, 3, 4, 6-pentagalloylglucose by enzymatic galloylation of 1, 2, 3, 6-tetragalloylglucose.* Archives of biochemistry and biophysics, 1989. **273**(1): p. 58-63.
242. Leela, V. and A. Saraswathy, *Isolation and characterization of phytoconstituents from Acacia leucophloea flowers (Roxb) wild.* Int Res J Pharm, 2013. **4**(9): p. 107-9.
243. Zhao, W.-H., et al., *The isolation of 1, 2, 3, 4, 6-penta-O-galloyl-beta-D-glucose from Acer truncatum Bunge by high-speed counter-current chromatography.* 2007. **850**(1-2): p. 523-527.
244. Yang, C., J.-G. Shi, S.-Y. Mo, and Y.-C.J.J.o.A.n.p.r. Yang, *Chemical constituents of Pyrrosia petiolosa.* 2003. **5**(2): p. 143-150.
245. Zapesochnaya, G. and T. Pangarova, *Hypolaetin 7-glucoside from Caryopteris monolica.* Chemistry of Natural Compounds, 1973. **9**(4): p. 521-521.
246. Choubey, S., L.R. Varughese, V. Kumar, and V. Beniwal, *Medicinal importance of gallic acid and its ester derivatives: a patent review.* Pharmaceutical patent analyst, 2015. **4**(4): p. 305-315.
247. Daglia, M., et al., *Polyphenols: well beyond the antioxidant capacity: gallic acid and related compounds as neuroprotective agents: you are what you eat!* Current Pharmaceutical Biotechnology, 2014. **15**(4): p. 362-372.
248. Kroes, B.v., et al., *Anti-inflammatory activity of gallic acid.* Planta medica, 1992. **58**(06): p. 499-504.
249. Hu, H., et al., *Penta-1, 2, 3, 4, 6-O-galloyl-β-d-glucose induces p53 and inhibits STAT3 in prostate cancer cells in vitro and suppresses prostate xenograft tumor growth in vivo.* Molecular cancer therapeutics, 2008. **7**(9): p. 2681-2691.
250. Kant, R., et al., *Induction of GNMT by 1, 2, 3, 4, 6-penta-O-galloyl-beta-D-glucopyranoside through proteasome-independent MYC downregulation in hepatocellular carcinoma.* Scientific reports, 2019. **9**(1): p. 1968.

251. Locatelli, C., F.B. Filippin-Monteiro, and T.B. Creczynski-Pasa, *Alkyl esters of gallic acid as anticancer agents: A review.* European journal of medicinal chemistry, 2013. **60**: p. 233-239.
252. Lee, H., et al., *Methyl gallate exhibits potent antitumor activities by inhibiting tumor infiltration of CD4+ CD25+ regulatory T cells.* The Journal of Immunology, 2010. **185**(11): p. 6698-6705.
253. Borges, A., C. Ferreira, M.J. Saavedra, and M. Simões, *Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria.* Microbial Drug Resistance, 2013. **19**(4): p. 256-265.
254. Buzzini, P., et al., *Antimicrobial and antiviral activity of hydrolysable tannins.* Mini reviews in medicinal chemistry, 2008. **8**(12): p. 1179-1187.
255. Choi, J.G., et al., *Methyl gallate from Galla rhois successfully controls clinical isolates of Salmonella infection in both in vitro and in vivo systems.* PLoS One, 2014. **9**(7): p. e102697.
256. Legault, J., et al., *Antioxidant and anti-inflammatory activities of quercetin 7-O-β-D-glucopyranoside from the leaves of Brasenia schreberi.* Journal of medicinal food, 2011. **14**(10): p. 1127-1134.
257. Shimoda, H., et al., *Anti-adipogenic polyphenols of water shield suppress TNF-alpha-induced cell damage and enhance expression of HAS2 and HABP2 in adiponectin.* Nat. Prod. Chem. Res, 2014. **2**(5): p. 146.
258. Braunberger, C., et al., *LC–NMR, NMR, and LC–MS identification and LC–DAD quantification of flavonoids and ellagic acid derivatives in Drosera peltata.* 2013. **932**: p. 111-116.
259. Veit, M., et al., *Interspecific and intraspecific variation of phenolics in the genus Equisetum subgenus Equisetum.* 1995. **38**(4): p. 881-891.
260. Barrow, R. and R. Capon, *Alkyl and alkenyl resorcinols from an Australian marine sponge, Haliclona Sp (Haplosclerida: Haliclonidae).* Australian Journal of Chemistry, 1991. **44**(10): p. 1393-1405.
261. Kozubek, A. and J.H. Tyman, *Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity.* Chemical reviews, 1999. **99**(1): p. 1-26.
262. Kozubek, A., R. Zarnowski, M. Stasiuk, and J. Gubernator, *Natural amphiphilic phenols as bioactive compounds.* Cellular and Molecular Biology Letters, 2001. **6**(2A): p. 351-355.
263. Tasdemir, D., et al., *Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, structure-activity relationship, and quantitative structure-activity relationship studies.* 2006. **50**(4): p. 1352-1364.
264. Koide, T., et al., *Trypanocidal effects of gallic acid and related compounds.* Planta medica, 1998. **64**(01): p. 27-30.
265. Nose, M., et al., *Formation of reactive oxygen intermediates might be involved in the trypanocidal activity of gallic acid.* Biological and Pharmaceutical Bulletin, 1998. **21**(6): p. 583-587.
266. Grady, R.W., E.J. Bienen, A.B.J.M. Clarkson Jr, and b. parasitology, *Esters of 3, 4-dihydroxybenzoic acid, highly effective inhibitors of the sn-glycerol-3-phosphate oxidase of Trypanosoma brucei brucei.* 1986. **21**(1): p. 55-63.
267. Amisigo, C.M., C.A. Antwi, J.P. Adjimani, and T.M.J.P.o. Gwira, *In vitro anti-trypanosomal effects of selected phenolic acids on Trypanosoma brucei.* 2019. **14**(5): p. e0216078.
268. Andjelković, M., et al., *Iron-chelation properties of phenolic acids bearing catechol and galloyl groups.* 2006. **98**(1): p. 23-31.
269. Wink, M.J.M., *Medicinal plants: a source of anti-parasitic secondary metabolites.* 2012. **17**(11): p. 12771-12791.
270. Jeacock, L., et al., *Aquaglyceroporin-null trypanosomes display glycerol transport defects and respiratory-inhibitor sensitivity.* PLoS Pathog, 2017. **13**(3): p. e1006307.

271. Andréo, R., et al., *Toxicity and loss of mitochondrial membrane potential induced by alkyl gallates in trypanosoma cruzi.* International scholarly research notices, 2015. **2015**.
272. Calderon, A.I., et al., *Evaluation of Larvicidal and in Vitro. Antiparasitic Activities of Plants in a Biodiversity Plot in the Altos de Campana National Park, Panama.* Pharmaceutical Biology, 2006. **44**(7): p. 487-498.
273. Khasanah, U., A. WidyaWaruyanti, A.F. Hafid, and M.J.P.r. Tanjung, *Antiplasmodial activity of isolated polyphenols from Alectryon serratus leaves against 3D7 Plasmodium falciparum.* 2017. **9**(Suppl 1): p. S57.
274. Arsianti, A., et al., *Synthesis and in Vitro Antimalarial Activity of Alkyl Esters Gallate as a Growth Inhibitors of Plasmodium Falciparum.* 2018. **34**(2): p. 655.
275. Torres-Leon, C., et al., *Pentagalloylglucose (PGG): a valuable phenolic compound with functional properties.* Journal of functional foods, 2017. **37**: p. 176-189.
276. Kolodziej, H., et al., *Antileishmanial activity of hydrolyzable tannins and their modulatory effects on nitric oxide and tumour necrosis factor-α release in macrophages in vitro.* Planta medica, 2001. **67**(09): p. 825-832.
277. Jin, W. and J.K.J.J.o.N.p. Zjawiony, *5-Alkylresorcinols from Merulius i ncarnatus.* 2006. **69**(4): p. 704-706.
278. Jiménez-Romero, C., et al., *Hydroxyalkenylresorcinols from Stylogyne turbacensis.* 2007. **70**(8): p. 1249-1252.
279. Belmonte-Reche, E., et al., *Tyrosol and hydroxytyrosol derivatives as antitrypanosomal and antileishmanial agents.* 2016. **119**: p. 132-140.
280. Matutino Bastos, T., et al., *Chemical Constituents of Anacardium occidentale as Inhibitors of Trypanosoma cruzi Sirtuins.* 2019. **24**(7): p. 1299.
281. Choo, L.M., et al., *Phylogeny and biogeography of the Daniellia clade (Leguminosae: Detarioideae), a tropical tree lineage largely threatened in Africa and Madagascar.* Mol Phylogenet Evol, 2020. **146**: p. 106752.
282. Mahmoud, A.B., et al., *Mining Sudanese Medicinal Plants for Antiprotozoal Agents.* Front Pharmacol, 2020. **11**: p. 865.
283. Oyeyemi, I.T., O.O. Akanni, O.A. Adaramoye, and A.A. Bakare, *Methanol extract of Nymphaea lotus ameliorates carbon tetrachloride-induced chronic liver injury in rats via inhibition of oxidative stress.* Journal of basic and clinical physiology and pharmacology, 2017. **28**(1): p. 43-50.
284. Xiao, H., X. Cai, Y. Fan, and A. Luo, *Antioxidant activity of water-soluble polysaccharides from Brasenia schreberi.* Pharmacognosy magazine, 2016. **12**(47): p. 193.
285. Adelakun, K.M., et al., *Phytochemical screening and antibacterial activities of crude extract of Nymphaea lotus (water lily) against fish pathogens.* Journal of Biomedical Sciences, 2015. **2**(4): p. 38-42.
286. Akinjogunla, O., C. Yah, N. Eghafona, and F. Ogbemudia, *Antibacterial activity of leave extracts of Nymphaea lotus (Nymphaeaceae) on Methicillin resistant Staphylococcus aureus (MRSA) and Vancomycin resistant Staphylococcus aureus (VRSA) isolated from clinical samples.* Ann Biol Res, 2010. **1**(2): p. 174-184.
287. Akinjogunla, O., A. Adegoke, I. Udokang, and B. Adebayo-Tayo, *Antimicrobial potential of Nymphaea lotus (Nymphaeaceae) against wound pathogens.* Journal of medicinal plants Research, 2009. **3**(3): p. 138-141.
288. Elakovich, S.D. and J.W. Wooten, *An examination of the phytotoxicity of the water shield, Brasenia schreberi.* Journal of chemical ecology, 1987. **13**(9): p. 1935-1940.
289. Kim, H., et al., *Polysaccharide gel coating of the leaves of Brasenia schreberi lowers plasma cholesterol in hamsters.* Journal of traditional and complementary medicine, 2015. **5**(1): p. 56-61.

290. Hisayoshi, T., et al., *Inhibition of the DNA polymerase and RNase H activities of HIV-1 reverse transcriptase and HIV-1 replication by Brasenia schreberi (Junsai) and Petasites japonicus (Fuki) components.* J Nat Med, 2015. **69**(3): p. 432-40.
291. Hisayoshi, T., et al., *Inhibition of HIV-1 reverse transcriptase activity by Brasenia schreberi (Junsai) components.* 2014. **14**(1): p. 59-65.
292. Tanaka, T., G. Nonaka, and I. Nishioka, *Tannins and Related-Compounds .14. 7-O-Galloyl-(+)-Catechin and 3-O-Galloylprocyanidin-B-3 from Sanguisorba-Officinalis.* Phytochemistry, 1983. **22**(11): p. 2575-2578.
293. Cui, C.B., et al., *Two new and four known polyphenolics obtained as new cell-cycle inhibitors from Rubus aleaefolius Poir.* Journal of Asian Natural Products Research, 2002. **4**(4): p. 243-252.
294. Yang, C., J.G. Shi, S.Y. Mo, and Y.C. Yang, *Chemical constituents of Pyrrosia petiolosa.* Journal of Asian Natural Products Research, 2003. **5**(2): p. 143-150.
295. Barrow, R.A. and R.J. Capon, *Alkyl and alkenyl resorcinols from an Australian marine sponge, Haliclona sp. (Haplosclerida: haliclonidae).* Aust. J. Chem., 1991. **44**(10): p. 1393-405.
296. Dozon, J.-P.J.P.a., *Ce que valoriser la médecine traditionnelle veut dire.* 1987(28): p. 9-20.
297. Yoro, B.M.J.R.q., *Rôle de l'anthropologue dans la revalorisation de la médecine traditionnelle africaine.* 2010. **29**(2): p. 57-67.
298. De Jonge, B.J.J.o.a. and e. ethics, *What is fair and equitable benefit-sharing?* 2011. **24**(2): p. 127-146.
299. Schroeder, D.J.J.o.m.e., *Benefit sharing: it's time for a definition.* 2007. **33**(4): p. 205-209.
300. Dauda, B. and S.J.D.w.b. Joffe, *The benefit sharing vision of H3Africa.* 2018. **18**(2): p. 165-170.
301. Bachmann, A., *Ethical aspects of Access and Benefit-Sharing (ABS). Commissioned by the Federal Office for the Environment FOEN. Zürich, March 2011.* 2011.
302. Zerda-Sarmiento, Á. and C.J.R.i.d.s.s. Forero-Pineda, *Les droits de propriété intellectuelle sur le savoir des communautés ethniques.* 2002(1): p. 111-127.
303. Willcox, M.L. and B. Gilbert, *Traditional medicinal plants for the treatment and prevention of human parasitic diseases.* 2009.
304. Tardío, J. and M.J.E.B. Pardo-de-Santayana, *Cultural importance indices: a comparative analysis based on the useful wild plants of Southern Cantabria (Northern Spain).* 2008. **62**(1): p. 24-39.
305. Willcox, M., et al., *Do ethnobotanical and laboratory data predict clinical safety and efficacy of anti-malarial plants?* 2011. **10**(1): p. S7.
306. Patwardhan, B. and A.D. Vaidya, *Natural products drug discovery: accelerating the clinical candidate development using reverse pharmacology approaches.* 2010.
307. Orhue, N., E. Nwanze, and A.J.A.J.o.F.S. Okafor, *Scoparia dulcis protects against Trypanosoma brucei-induced immunosuppression in experimentally infected rabbits.* 2009. **3**(6): p. 172-176.
308. Peltzer, K., N. Friend-du Preez, S. Ramlagan, and H.J.B.p.h. Fomundam, *Use of traditional complementary and alternative medicine for HIV patients in KwaZulu-Natal, South Africa.* 2008. **8**(1): p. 255.
309. Langlois-Klassen, D., et al., *Use of traditional herbal medicine by AIDS patients in Kabarole District, western Uganda.* 2007. **77**(4): p. 757-763.
310. Peltzer, K., et al., *Antiretrovirals and the use of traditional, complementary and alternative medicine by HIV patients in Kwazulu-Natal, South Africa: a longitudinal study.* 2011. **8**(4).
311. Hamoud, R., S. Zimmermann, J. Reichling, and M.J.P. Wink, *Synergistic interactions in two-drug and three-drug combinations (thymol, EDTA and vancomycin) against multi drug resistant bacteria including E. coli.* 2014. **21**(4): p. 443-447.

312. Stepanović, S., N. Antić, I. Dakić, and M.J.M.R. Švabić-Vlahović, *In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs.* 2003. **158**(4): p. 353-357.
313. Hatano, T., et al., *Theasinensin A, a tea polyphenol formed from (-)-epigallocatechin gallate, suppresses antibiotic resistance of methicillin-resistant Staphylococcus aureus.* 2003. **69**(11): p. 984-989.
314. Akiyama, H., et al., *Antibacterial action of several tannins against Staphylococcus aureus.* 2001. **48**(4): p. 487-491.
315. Ulrich-Merzenich, G.S.J.S., *Combination screening of synthetic drugs and plant derived natural products—Potential and challenges for drug development.* 2014. **1**(1): p. 59-69.
316. Ahmad, I., F. Aqil, and M. Owais, *Modern phytomedicine: Turning medicinal plants into drugs*. 2006: John Wiley & Sons.
317. Taylor, J., et al., *Towards the scientific validation of traditional medicinal plants.* 2001. **34**(1): p. 23-37.
318. Gertsch, J.J.J.o.e., *How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems.* 2009. **122**(2): p. 177-183.
319. Patwardhan, B. and R.A.J.D.d.t. Mashelkar, *Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward?* 2009. **14**(15-16): p. 804-811.
320. Willcox, M.L., et al., *A "reverse pharmacology" approach for developing an anti-malarial phytomedicine.* 2011. **10**(1): p. S8.
321. Simoes-Pires, C., et al., *Reverse pharmacology for developing an anti-malarial phytomedicine. The example of Argemone mexicana.* 2014. **4**(3): p. 338-346.
322. World Health Organization, *Exepert Committee on specifications for pharmaceutical preparations: Thirthy-fourth report. WHO, Geneva.* 1996.