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Ecology, conservation and bioactivity of food and medicinal plants in East Africa

Økologi, bevaring av og bioaktivitet i mat og medisinplanter i Øst-Afrika

Torunn Stangeland

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Paper VI *Stangeland T*. Plants used to treat malaria in Nyakayojo sub-county, Western Uganda.

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ABSTRACT

In East Africa 70-80 % of the population is subsistence farmers. The rural health system is often poorly equipped both with personnel and medicines, and many depend on the use of medicinal plants for their primary health care. Malaria is the single most serious cause of morbidity, mortality and poverty, partly because the parasite causing the disease, *Plasmodium falciparum,* has developed resistance towards the most common and affordable medicines, and the fact that a large part of the population is malnourished. In East Africa many plants are used in the treatment of malaria, but most of them are poorly investigated for effect and safety. Bark and roots of trees are often used for medicine, and some of the trees are locally threatened because of population increase and deforestation.

Traditional medicine has received increased attention from governments in Tanzania and Uganda. In Tanzania laws and regulations on management of natural resources and traditional medicine, which are in line with the Convention on Biological Diversity, are now in place. However, important ecosystems for medicinal plants are heavily degraded. In Uganda laws and regulations for traditional medicine are now under debate in the government.

Experiments on germination and seedling growth for two Ugandan medicinal trees, *Mitragyna (Hallea) rubrostipulata* and *Sarcocephalus latifolius,* was conducted in controlled environments. Both needed light to germinate, *Mitragyna rubrostipulata* had a temperature optimum at 25 ºC with 79 % germination, while germination for *Sarcocephalus latifolius* after 28 days was around 60 % for the temperatures 20-35 ºC. A germination field experiment failed, indicated that these species need assistance from nursery to be able to establish in degraded areas.

The framework tree species method was chosen to conserve and gain more knowledge about local medicinal trees. In this method 25-40 different local woody species are raised in nursery and planted in a single event in a mixed stand. The intention is to encourage regeneration of degraded forest. We raised and planted 27 mainly indigenous woody species in three plots in April 2008, and monitored survival and growth for 13 months. Eleven species turned out to be excellent framework species, while eight others were acceptable. Some of the important medicinal trees we failed to cultivate, partly because they have become so rare that we did not find seeds.

Total antioxidant activity (AOA) in 35 Ugandan fruits and vegetables were measured using the Ferro Reducing Ability of Plasma (FRAP) method. The results showed large variation in AOA from 72.5 ± 13.5 (*Syzygium cuminii* seeds) to 0.09 ± 0.05 (*Curcubita maxima* fruit) mmol/100 g fresh weight. Antioxidant activity per serving was calculated, and the food with heighest AOA per serving were pomegranat (*Punica granatum*)*, Canarium schweinfurthii,* guava (*Psidium guajava),* mango (*Mangifera indica*) and tree tomato (*Cyphomandra betacea*) with values from 8.91 to 3.00 mmol/serving. AOA for Ugandan mango in this study was five times higher than values found in another study of mangoes bought in Norway. In Uganda the intake of antioxidants can be relative easily increased by adding more of the fruits that are abundant in the fruiting seasons and green leafy vegetables.

Raw extracts from tree medicinal plants that are used to treat malaria in Uganda were tested for antioxidant (DPPH, FRAP, Total phenols) and anti-plasmodial activity. The water extract of *Mitragyna rubrostipulata* showed highest anti-plasmodial activity (IC_{50}= 1.95 µg/ml), and high antioxidant activity as well. Thirteen other extracts showed high anti-plasmodial activity ranging from 2.12 to 3.63 µg/ml (chloroquine control: IC_{50}= 8 µg/ml). There was high correlation between the different antioxidant assays.

In March – April 2009 twenty-eight traditional birth attendants and healers from Mbarara District in Western Uganda were interviewed about how they use plants to treat malaria. Altogether they used 57 different plant species from 27 families. Asteraceae was most common, with 17 species used, followed by Fabaceae (8) and Lamiaceae (5). Leaves were the most commonly used plant part, and in most cases fresh leaves were either pounded and juice squeezed out, or decoction were made for oral intake. More than 80 % of the recipes included *Vernonia amygdalina,* a species used for treatment of malaria all over Africa, and for which effect has been documented.

SAMMENDRAG

I Øst Afrika er 70-80 % av befolkningen selvforsyningsbønder. Det lokale helsesystemet er ofte dårlig bemannet og utstyrt, og mange baserer seg på den tradisjonelle bruken av medisinplanter for å behandle vanlige sykdommer. Malaria er den viktigste enkeltårsaken til sykdom, død og fattigdom, delvis fordi parasitten som forårsaker sykdommen har utviklet resistens overfor de vanligste og rimeligste medisinene, og fordi en stor del av befolkningen er under- eller feilernært. Mange planter blir brukt til behandling av malaria, men de fleste er dårlig undersøkt for effekt og sikkerhet. Bark og røtter av trær brukes ofte medisinsk, og noen trær er lokalt truet på grunn av befolkningsøkning og avskoging.

I Tanzania og Uganda har den tradisjonelle medisinen fått økende oppmerksomhet fra myndighetene. Tanzania har nå lovverket på plass for forvalting av natur ressurser og praktisering av "Traditional Medicine" i samsvar med Konvensjonen om Biologisk Mangfold, mens i Uganda er fortsatt disse lovene under debatt. Viktige økosystem for medisinplanter er ødelagte.

Spirings- og vekstforsøk for to medisinske trær, *Sarcocephalus latifolius* og *Mitragyna (Hallea) rubrostipulata* ble utført i kontrollerte omgivelser. Begge trengte lys for å spire, *Mitragyna rubrostipulata* hadde temperaturoptimum for 25 ºC med 79 % spiring, mens spiring for *Sarcocephalus latifolius* etter 28 dager var rundt 60 % for temperaturene 20-35 ºC. Såforsøk i felt var mislykket og tyder på at gjenetablering av disse artene i degraderte områder er avhengig av oppal i planteskole.

"The framework tree species method" ble valgt for å bevare og få bedre kjennskap til lokale medisinske trær. Denne metoden innebærer oppal og utplanting av 25-40 forskjellige treslag samtidig med den hensikt å fremme regenerering av skog. Vi plantet ut 27 hovedsakelig lokale treslag i tre felt i april 2008, og registrerte overlevelse og vekst i et år. Elleve arter viste meget god overlevelse og vekst, mens åtte flere hadde akseptabel vekst. Noen viktige medisinske trær har vi foreløpig ikke klart å oppformere, delvis fordi de har blitt så sjeldne at vi ikke klarte å finne frø.

Total antioksidant aktivitet (AOA) i 35 ugandiske frukt og grønnsaker ble målt ved hjelp av FRAP (Ferro Reducing Ability of Plasma). Resultatene vist stor variasjon i AOA fra 72,5 ± 13,5 (*Syzygium cuminii* frø) til 0,09 ± 0,05 (*Curcubita maxima* frukt) mmol/100 g fersk vekt.

Antioksidant aktivitet per porsjon ble kalkulert, og de matvarene som hadde høyest AOA per porsjon var granateple (*Punica granatum)*, *Canarium schweinfurthii,* guava *(Psidium guajava),* mango *(Mangifera indica)* og tretomat *(Cyphomandra betacea)* med verdier fra 8,91 til 3,00 mmol/porsjon. AOA for Ugandisk mango i dette forsøket var fem ganger så høy som verdier målt i mango kjøpt i Norge. Inntak av antioksidanter kan relativt lett økes ved å innta mer av de matvarene som har høy AOA, og som mange steder er tilgjengelige i overflod i fruktsesongen.

Råekstrakter fra tre medisinplanter (*Mitragyna rubrostipulata, Vernonia adoensis and Zanthoxylum chalybeum),* som brukes i Uganda til behandling av malaria, ble testet for antioksidant (DPPH, FRAP, Total fenoler) og anti-plasmodial aktivitet. Vi fant at vannekstraktet av *Mitragyna rubrostipulata* hadde høyest anti-plasmodial aktivitet (IC_{50}= 1,95 µg/ml), samt høy antioksidant aktivitet. Tretten andre ekstrakter hadde høye anti-plasmodiale aktiviteter med verdier mellom 2,12 til 3,63 µg/ml (klorokin kontroll: IC_{50}= 8 µg/ml). Vi fant høy korrelasjon mellom de forskjellige antioksidant-testene.

I mars - april 2009 ble 28 tradisjonelle fødselshjelpere fra Mbarara distrikt i det vestlige Uganda intervjuet om hvordan de bruker planter for å behandle malaria. De brukte til sammen 57 forskjellige arter fra 23 familier. Korgplantefamilien var den vanligste, med hele 17 arter, dernest erteblomstfamilien (8) og leppeblomstfamilien (5). Blader av urtene var den vanligste plantedelen brukt, og i de fleste tilfeller ble enten friske blader knust og plantesaften presset ut, eller det ble laget avkok av ferske eller tørkede plantedeler. Over 80 % av oppskriftene innbefattet *Vernonia amygdalina,* som er brukt til behandling av malaria over store deler av Afrika, og hvor effekt er dokumentert.

Errata

Introduction

References to figures in the text are added in the introduction on pages 7, 10, 14, 16, 20, 21, 25, 26 and 27.

Page 36, 4.3., line 3:

'total antioxidant activity' is changed to 'total dietary antioxidant capacity'

Page 37, 4.4., line 11:

'Z. chalybeum' is changed to '*V. adoensis'*

Page 37, 4.5., line 1:

'56' is changed to '57'

Paper V:

Page 4, left side, line 5, after (...1994). : This text is added: '*V. adoensis* has not previously been tested for anti-plasmodial activity. We found that all extracts had high anti-plasmodial activity ranging from IC_{50} 2.14 – 2.83 µg/ml.

The next sentence (p 4, line 8) is changed to: 'Four extracts of *Z. chalybeum* had good activity as well (…); only the methanol extract had low activity (..).'

Page 8, Conclusion, line 19: '*Z. chalybeum'* is changed to '*V. adoensis'*

1. INTRODUCTION

Plants are the primary producers that all life on earth depends on. In all cultures people have used plants to stay healthy and to treat illnesses. Thousands of years of experience developed into different health systems; some were oral traditions like most African, and some documented in writing like the Chinese, Indian and Egyptian. The Western medical system was developed over time from Greek tradition. The famous physician Hippocrates (400 BC) is regarded as the father of Western medicine. He described many medicinal plants and introduced the Hippocratic Oath. In the first century AC Dioscorides worked as a Roman military surgeon under Nero, and was able to travel all over the Roman and Greek world. He collected information about medicinal plants and wrote down De Materia Medica (about medical matter); where over 500 plants were described botanically and medicinally. It is regarded as one of the most influential herbal books in history, and was in use for more than 1000 years (Gurib-Fakim 2006).

In modern European research there was a shift in emphasis around the beginning of the 19th century, when it became clear that the medicinal effect of a plant was due to specific compounds that were possible to isolate and characterise. Morphine was isolated from opium poppy (*Papaver somniferum* L.*)* in 1805 and Quinine from cinchona bark (*Cinchona* spp.) in 1820. The new science of Phytochemistry developed rapidly during the following century, and by the end of the century another development occurred: syntheses of compounds. The first successful use of synthetic compound for therapeutic use was achieved by Paul Ehrlich, who developed methylene blue as a treatment of malaria in 1891 (Heinrich et al. 2004). For a while it was believed that all illness could be cured by synthesizing new medicine. However this optimism was set back by the thalidomide scandal after the Second World War, resulting in a renewed interest in finding useful plants and plant compounds. New technology for bioactivity screening, isolation and characterization of natural compounds has developed during the last decades. Several Pharmaceutical companies have taken part in the screening and development of new medicines based on compounds found in plants: vincristine and vinblastine from *Catharanthus roseus* G.Don, d-Tubocurarine from *Chondrodendron tomentosum* and sitosterol from *Prunus africana* (Hook.f.) Kalkman*,* all developed into important 'modern' medicines (Heinrich et al. 2004). This activity also generated criticism: companies from the North used natural resources

from the South to develop medicines for people in the North, without returning any benefits to the people and countries that provided the knowledge and the plant material. The injustice became apparent, and in 1992 regulations on Prior informed Consent (PIC) and Access and Benefit Sharing (ABS) were incorporated in the Convention of Biological Diversity (CBD). 'The Convention on Biological Diversity was inspired by the world community's growing commitment to sustainable development. It represents a dramatic step forward in the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of benefits arising from the use of genetic resources.' (CBD 2010). CBD states that all nations have sovereignty over its own natural resources. If anyone wants to do research on natural resources, they must get PIC: the people in the area of study must be informed about the aim and methods of the study, and give their consent to the activity before it starts. The regulations on ABS give citizens and companies from developed countries access to natural resources in developing countries on certain conditions. One of them is that if benefits are achieved through the access, they shall be shared with the local community or government. Today 193 countries have ratified CBD, but in order to be operational there must be national legislation in line with CBD, which for many countries are not the case. For Tanzania the national legislation is operational, but for Uganda it is still under debate.

The World Health Organization (WHO) has encouraged i) the use of safe, effective and quality products and services from traditional medicine, ii) the developing countries to support and integrate traditional medicine into national health systems, and iii) that knowledge and resources must be preserved (WHO 2008). Most medicinal plants are not analysed or tested for efficacy and safety, but many developing countries lack equipment and capacity to do these analysis. Cooperation between researchers in the North and South is needed, and laboratories in Northern countries should be made available to ease the development of Improved Traditional Medicine, like they have done in Mali (Diallo et al. 2004, Nergard et al. 2004).

However, there is no use in finding out that a plant is safe and effective, if it is in danger of being extinct. Actually, such information could increase the pressure on the wild resources, and accelerate extinction. That is why it is important to work on conservation methods for plants in

high demand. In this study we have concentrated on finding a way to cultivate locally threatened medicinal plants.

'Let your food be your medicine and medicine your food' is a famous quotation from Hippocrates, which have been revitalised among others by the study of antioxidants, and the renewed interest and sale of nutraceuticals in Western countries. This knowledge and interest has sadly not reached East Africa, where people's food habits the last decades have undergone a major transition from traditional local whole foods to easy-to-prepare foods bought at supermarkets (Raschke & Cheema 2008). The globalised food system is gradually changing the traditional food habits in East Africa, which has many health benefits, and is fostering increased consumption of refined flour and sugar, inexpensive vegetable fats and food additives, which are known to hasten the development of non-communicable diseases such as cancer, diabetes and cardiovascular diseases. In this way many developing countries have come under a double burden of disease; under- and malnutrition alongside with emerging chronic diseases and obesity (WHO/FAO 2003). Hunger and malnutrition is still the number one risk to health worldwide (WFP 2010). The number of undernourished people in the world increased by 75 million in 2007, and by 40 million in 2008, largely due to higher food prices (FAO 2008). This show that the international financial crisis have had a detrimental effect also on developing countries, and made it even worse to reach the Millennium Development Goals (MDG), especially number 4 and 5: reduction of child mortality and improvement of maternal health. Hunger is closely connected to poverty, but as money and land are scarce it is even more important to target what to grow and eat. Several fruits and vegetables that can improve nutritional status, like mango, papaya, passion fruits and the green leafy vegetables, are already available. But information and campaigns are needed to make people aware of the importance of these foods.

In Uganda malaria is the number one cause of death, and the disease especially hits pregnant mothers and children below five years of age. Recent research point at malnutrition and certain deficiencies, especially iron, vitamin A and zinc as responsible for a substantial portion of malaria morbidity and mortality (Villar et al. 2003, Caulfield et al. 2004, Lartey 2008). The disease has become more severe in East Africa, as the *Plasmodium falciparum* parasite has developed resistance towards the most common and affordable medicine: chloroquine. The new

artemisinin based combination medicine is now recommended by most countries, but it is far more expensive, and in many rural areas not available. Resistance towards artemisinin is detected in Asia, and WHO emphasise the importance of finding new compounds that can fight the disease (WHO 2009).

This study is a continuation of a NUFU cooperation project on medicinal plants between the Norwegian University of Life Sciences and Makerere University in Uganda from 2000 to 2005. In the NUFU project the diversity and local use of plants in Bulamogi County, Kaliro District (Tabuti et al. 2003), plants to treat malaria in Sango Bay area (Ssegawa and Kasenene 2007a) and Mbarara district (Katuura et al. 2007) were among the projects. Previous work revealed serious degradation of the environment (Tabuti 2007; Tabuti et al. 2009) and some of the medicinal plants were overharvested (Tabuti 2007, Ssegawa and Kasenene 2007b).

1.1. Ethnobotany and Ethnopharmacology

Ethnobotany is the study of how people in different cultures classify, manage and use plants for food, building material, energy, fodder, ceremonies, religion and medicine. According to Martin (1995) 'there are four basic interrelated endeavours in ethnobotany: (1) basic documentation of traditional botanical knowledge; (2) quantitative evaluation of the use and management of botanical resources; (3) experimental assessment of the benefits derived from plants, both for subsistence and for commercial ends; and (4) applied projects that seek to maximize the value that local people attain from their ecological knowledge and resources.' The research is interdisciplinary, the most important fields of study being botany, ethnobotany, natural resource management, anthropology, ecology, economics and linguistics. The term "Ethnobotany" was used for the first time by the US botanist J. W. Harchberger in 1895. Today the study is focusing on the interrelationship between people and plants, particularly the way in which plants impact on human culture and practices, how humans have used and modified plants, and how they represent them in their systems of knowledge (University of Kent, 2010).

Ethnopharmacology is broadly defined as a 'multi-disciplinary area of research, concerned with the observation, description and experimental investigation of indigenous drugs and their

biological activities' (Rivier & Bruhn 1979). Ethnopharmacology is a young and fast developing area of research. The term was first used in 1967 in the context of the study of hallucinogenic plants (Society of Ethnopharmacology, 2010). Field based ethnopharmacological studies have until now often had a descriptive focus. It is now widely accepted that scientific research should be hypothesis driven, and there is now an urge to change focus in this direction for ethnopharmacology as well (Heinrich et al. 2009).

The first paper in this study is a review giving background information about the situation for traditional medicine and natural resource management in East Africa, with Tanzania as an example. In Paper II, III and IV we have used an ethnobotanical, and in Paper V and VI an ethnopharmacological approach. Field sites have been in Uganda.

1.2. Ecology and conservation of medicinal plants

In many African and Asian countries up to 80% of the population rely on traditional medicine for their primary health care. Herbal treatments are the most popular form of traditional medicine and generate high revenues on the international market place (WHO 2008). When traditional medicine is used in another culture it is often called complementary or alternative medicine (CAM). In many developed countries 70-80 % of the population has used some form of CAM, and herbal medicine has become increasingly popular the last decades. In addition 25% of prescription drugs in allopathic or "modern" medicine are derived directly from flowering plants or modelled on plant molecules (Hawkins 2008). Most medicinal plants are harvested in the wild. The human population is still increasing rapidly, especially in Africa South of Sahara (ASS). This coupled with destructive harvesting practices, degradation of forest, agricultural expansion, grazing pressure and urbanisation threatens the survival of medicinal plants. The latest development is that rich persons and countries lease land in Africa to grow food for its own population. It is estimated that total area leased or bought by foreigners for this purpose by April 2010 is twice the size of Great Britain (Vidal 2010). This will put a new pressure on land and biodiversity. About 70000 plant species worldwide are used medicinally, and as many as 15000 of them may be under threat (Hawkins 2008).

We wanted to use the Framework tree species method (Blakesley et al. 2002, Elliott et al. 2003) as a way to conserve and secure supply of important and locally threatened medicinal woody species. Framework species are indigenous tree species planted in a mixed stand to accelerate natural regeneration of forest and encourage biodiversity regeneration. In this study we used the framework species method to make multipurpose tree gardens to provide traditional healers with woody species used for medicine and other needs like food and firewood.

1.3. Plant antioxidants and human health

1.3.1. Malnutrition and the importance of food for health

Today it is estimated that 1 in 6 people in the world do not get enough food to be healthy. Hunger and malnutrition lead to unsustainable use of natural resources, weakened immune system and increased maternal and infant mortality (Caulfield et al., 2004; Lartey, 2008). It is estimated that maternal and child undernutrition is the underlying cause of 3.5 million deaths annually, 35% of the disease burden in children younger than 5 years and 11% of total global disability-adjusted life-years (DALYs). In an overview of effectiveness and nutritional interventions to prevent or treat maternal morbidity, mortality and preterm delivery, Villar et al. (2003) conclude that until more research is done, women and their families should receive support to improve their diets as a general rule, which is a basic human right. For some years there has been little attention from health authorities and international donors on the importance of nutrition in developing countries. This seems to change now. Academy for Educational Development (AED) and USAID's African Bureau are now addressing nutritional and food security problems by delivery of Essential Nutrition Actions (ENA) into maternal, newborn, and child health programs (AED 2010). In fact Africa South of Sahara is the continent with least consumption of vegetables (WHO/FAO 2003). This situation together with the fact that land is getting scarce in many developing countries, population is increasing, and land is being leased by foreigners make it even more important to target what the families grow and eat toward a more adequate diet.

We have analyzed fruits and vegetables growing in Uganda for antioxidants, and found that there are several good sources of antioxidants and other essential nutrients and micronutrients available at least in the fruiting seasons. One challenge is to bring information about the importance of including more fruits and vegetables in the diet. Another is how to conserve large fruit harvests for use the rest of the year.

1.3.2. Oxidative stress

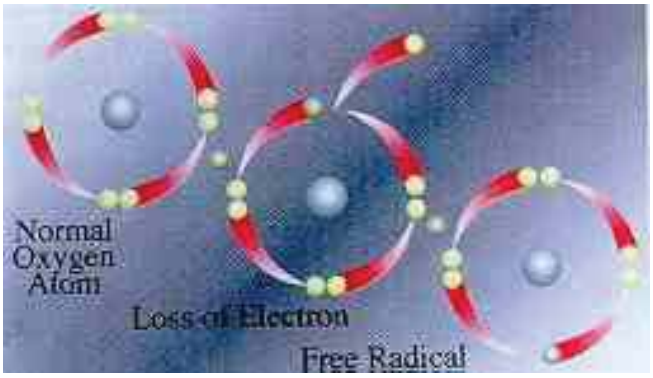

Fig. 1. Formation of a free radical

A free radical is any species capable of independent existence that contain one or more unpaired electron(s) (Halliwell 2005) (Fig. 1). Free radicals and other reactive oxygen species (ROS) are constantly generated in the human body as a result of oxidative metabolism, several diseases, and external sources like environmental poisons, alcohol, smoking and ionizing radiation (Blomhoff et al. 2006). The body has an antioxidant defense, but if the production of ROS is too high compared with the antioxidant defense there will be an imbalance, called oxidative stress. Oxidative stress is associated with the development of a wide range of diseases.

1.3.3. Antioxidants

Antioxidants are defined as any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell 2005). Antioxidants are widely used in food, pharmaceutical and cosmetic industry to prevent oxidative reactions. However, recent interest in antioxidants is caused by the assumption that they might have preventive effect on the development of cancer, heart and inflammatory diseases and aging processes.

There are four general sources of antioxidants: (1) enzymes like superoxide dismutase, glutathione peroxidase, catalase; (2) large molecules (albumin, ferritin and other proteins); (3) small molecules (ascorbic acid, glutathione, uric acid, tocopherol, carotenoids, polyphenols); and some hormones (estrogens and melatonin etc.) (Prior et al. 2005). Several antioxidants are produced in the body, but it is believed that the antioxidant defence can be strengthened by dietary antioxidants. This has been explained by a network of antioxidants with different chemical properties that may work together in a synergistic way, protecting the cells from damage (Blomhoff et al. 2006). The most common antioxidants in fruits and vegetables are vitamin C and E, selenium, carotenoids and phenolic compounds (Lindsay & Astley 2002). Carotenoids are lipophilic pigments that are synthesised in plants only, and there are at least 60 different carotenoids in fruits and vegetables consumed. The pro-vitamin A carotenoids are the main source of vitamin A activity in humans. In Africa vitamin A is one of the most serious micronutrient deficiencies, together with iron, iodine and zinc coming up as an increasing concern. Zink is not an antioxidant per se, but is a vital component in numerous antioxidant enzymes. Fruits, including berries and nuts, some seeds, vegetables, and some beverages (coffee, tea, red wine and fruit juices) are good antioxidant sources.

1.3.4. Methods for determining antioxidant activity (AOA)

There are multiple free radical and oxidant sources, and different antioxidants will respond in different manner to different radical or oxidant sources (Prior et al. 2005). For example carotenoids are not very good quenchers of peroxyl radicals compared to phenolics but are

exceptionally good at quenching singlet oxygen. Many reactions and mechanisms are usually involved in an antioxidant system, and no single assay will accurately reflect all of the radical sources or all antioxidants in a mixed complex system. That is why there is no simple universal method by which AOA can be measured accurately, and why it is recommended to use several methods parallel.

DPPH

The DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay is based on the reduction of stable free DPPH radical that absorbs at 517 nm. Results are expressed as the half minimum inhibition concentration (IC_{50}), which is the concentration at which half of the free radicals are scavenged. The method is simple and rapid and need only a UV-vis spectrophotometer to perform. However Prior et al. (2005) argue that the method has drawbacks: some antioxidants like carotenoids have spectra that overlap DPPH at 515 nm, and DPPH can act both as a radical probe and an oxidant. Thus AOA is not fairly rated by the ability of antioxidants to react with DPPH.

FRAP

The ferric reducing ability of plasma (FRAP) assay (Benzie and Strain 1996) is originally developed to measure antioxidant power of plasma, but has been extended to other biological fluids and food stuffs. The method is based on a sample's ability to reduce a Fe^{3+}complex to the intensively blue coloured Fe^{2+} complex. The method is simple, speedy, inexpensive, and robust and does not require specialised equipment. The FRAP method can be performed automatic, semiautomatic or by manual methods. However the method has some drawbacks: it does not measure thiol antioxidants, such as glutathione, and it only measures the reducing capacity on the iron ion, which is not relevant to antioxidant activity mechanistically or physiologically (Prior et al. 2005).

Folin-Ciocalteu (F-C)

The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent. Basic

mechanism in the F-C or total phenolics assay is an antioxidant/reduction reaction. It has been used to measure total phenols, but it measures other reducing agents as well, and can be considered as another AOA method. The method is simple, sensitive and precise (Prior et al. 2005). Gallic acid is used as a reference standard, and the results are given as gallic acid equivivalents.

1.4. Malaria

Malaria is a life threatening disease caused by a *Plasmodium* parasite, transmitted to humans through the bite of a female *Anopheles* mosquito (Fig. 2). There are around 300 million cases of malaria worldwide each year, and nearly 1 million die from the disease (WHO, 2009). Ninety percent of these deaths occur in Africa south of Sahara (ASS), and most of them are children under the age of five. In Africa today malaria is understood as both a disease of poverty and a cause of poverty (Sachs & Malaney 2002).

Malaria is preventable and curable, but in most of the seriously infected areas (ASS) there has been little success in fighting the disease (WHO, 2009) due to the parasite developing resistance to affordable medicines, poverty and ineffective health systems. However during the last years some areas and countries like Zanzibar, Eritrea, Rwanda and Zambia have managed to reduce recorded cases and deaths by 50%, suggesting that the Millennium Development Goals targets can be achieved if there is adequate coverage of key interventions. In these countries insecticide-treated nets (ITN) have been distributed to around 60-75% of households, compared to 16% in Uganda (WHO, 2009) and the treatment of cases have been intensified. The international disbursement to malaria-endemic countries (UD$ 0.65 billion in 2007) is still far from the US$ 5 billion required annually to ensure high coverage and maximal impact worldwide according to WHO (2009).

There are four types of parasites infecting people: *Plasmodium falciparum, P. vivax, P. malariae* and *P. ovale. Plasmodium falciparum* is the most deadly, and in Africa by far the most common parasite, causing 98% of cases (WHO, 2009). In humans the parasites first grow and multiply in the liver cells and then in the red blood cells (erythrocytes). In the blood successive broods of

parasites grow inside the erythrocytes and destroy them, releasing daughter parasites that continue the cycle by invading new red blood cells. The blood stage parasites are those that cause the symptoms of malaria, and it is also in this stage the infection can be demonstrated by a microscopy smear test (Center for Disease Control and Prevention, 2010). When sexual forms of blood stage parasites are picked up by a female *Anopheles* mosquito during a blood meal, a new cycle of parasite development and multiplication is started within the mosquito. After 10-18 days the parasite can be found in the mosquito's salivary gland and are ready to be injected into a new human, thus carrying the disease on. In *Plasmodium vivax* and *P. ovale* a dormant stage can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.

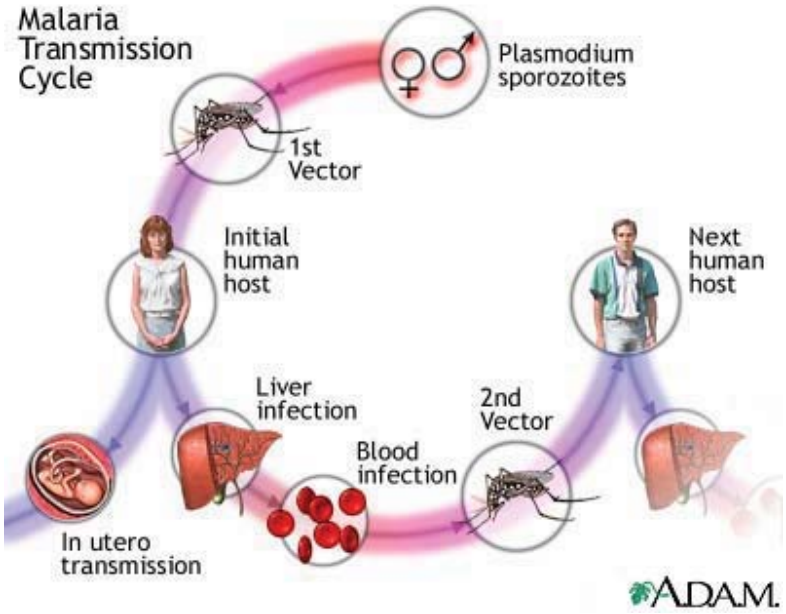

Fig. 2. Malaria Transmission Cycle

When the parasite develops in the erythrocyte, numerous waste substances, also toxic ones accumulate in the infected red blood cells. When the blood cells lyse and release new parasites, the waste products are dumped into the blood stream which acts to produce fever and probably influence other severe conditions. *Plasmodium falciparum* infected red blood cells, particularly those with mature parasites, adhere to the vascular endothelium of venular blood vessel walls

and do not freely circulate in the blood. When this happens in the blood vessels of the brain it is believed to be a factor in causing cerebral malaria, which is associated with high mortality (Centers for Disease Control and Prevention, 2010).

Chloroquine (CQ) and the combination drug of sulphadoxine–pyrimethamine (SP) were, until recently, the main malaria treatment in Africa. Resistance to both drugs is now widespread (Pears et al. 2009). CQ was first used in the 1950s, and chloroquine resistance (CQR) appeared in Asia and South America in the early1960s. CQR did not appear in Africa until 1978, when the initial focus was in East Africa. It subsequently reached West Africa at the end of the 1980ies. Artemisinin-based combination therapy (ACTs) is now the recommended and in many cases the only effective treatment of *Plasmodium falciparum* malaria. By 2009, 77 of 81 *P. falciparum* malaria-endemic countries had adopted ATCs for use in their drug policy. However in 11 of 13 African countries surveyed in 2007-2008, fewer than 15% of children below 5 years of age with fever had received an ACT, far below the WHO target of 80% (WHO, 2009). WHO is monitoring the global supply and demand of the artemether-lumefatrine fixed-dose combination, as part of a memorandum of understanding with the manufacturer Novartis in 2001 to make Coartem® available at cost price in the public sector. In public sale the medicine is far more expensive than the traditional chloroquine, and in most rural areas in Uganda it was not even available in 2009.

Artemisinin is extracted from *Artemisia annua* L.*,* a Chinese medicinal plant used against fever from old times, but 'rediscovered' in the 1970ies, when Mao intensified the fight against malaria. It is now grown in many African countries, including Tanzania and Uganda, and factories to produce medicine are established in both countries. However the expansion of agricultural production of *Artemisia annua* and extraction of artemisinin were not matched by artemisinin demand by the ATC producers in 2006-2007, resulting in a surplus and very low prises on Artemisia raw product, even below production costs. The subsequent withdrawal of producers and extractors from the marked is likely to create shortage of artemisinin-based active ingredients in 2010, when demand for ATC is expected to increase, partly because of increased mobilization of funds from international donors (WHO, 2009). There have already been some

reports on resistance towards artemisinin, and WHO is requesting pharmaceutical companies to stop producing oral monotherapy artemisinin products, which are still sold in some countries.

1.5. Medicinal plants and bioactivity

Bioactive principles can be primary or secondary metabolites in plants. The primary metabolites are compounds ubiquitous and essential for life, such as carbohydrates, essential amino acids and proteins (EEA 2010). Secondary metabolites are chemicals produces by plants, for which no role in primary functions for life have been found. They are an extremely diverse group of compounds, and are found in small amount in different plant parts. Many secondary compounds act as anti herbivore and/ or defence against pathogens and there is a hypothesis that the compounds are developed to protect the plants from enemies. Although polysaccharides are found to play important role as immunomodulators (Nergard et al. 2004) it is mainly the secondary metabolites that are used by humans to treat illnesses and to make medicines.

The secondary metabolites can be roughly grouped in three: (1) the terpenes, which are made from mevalonic acid and mainly contain carbon and hydrogen; (2) the phenolics which are made from simple sugars and consist of benzene rings, hydrogen and oxygen; and (3) the alkaloids that contain nitrogen in addition to carbon, hydrogen, oxygen and occasionally sulphur (EEA 2010).

However recent research have reviled important ‘primary’ roles for these compounds in the plants, as signalling molecules and antioxidants, so as we learn more we may find that they are not ‘secondary’.

Plant families and species in this study

Both in the conservation and the antioxidant study we worked with a wide range of plant species. However, there are a few plants that were studied in more detail in the germination study (Paper II) and the study of malaria plants (Paper V). Descriptions of these plants, their families, and previous relevant research on the species are described in the following text.

1.5.1. Rubiaceae

Rubiaceae (coffee family) is a big family of about 500 genera and 6000 species with a mostly pantropical and subtropical distribution; in East Africa 109 genera and 740 species (Lye et al. 2008). The family is recognised by simple leaves, prominent interpetiolar stipules, inferior ovaries and gamopetalous flowers. It is known for a large diversity of natural products, including iridoids, alkaloids, methylxanthines (caffeine, theobromine and theophylline) and anthranoids (Heinrich et al. 2004). Evidences from molecular phylogenies (Bremer 1996, Razafimandimbison & Bremer 2002) suggest that the family can be divided into three subfamilies (Cinchonoideae, Ixoroideae, and Rubioideae). The previously most commonly used malaria medicine, chloroquine, is derived from the bark of *Cinchona officinalis* L. and related species. The genus *Cinchona* belongs to tribe Cinchoneae in subfamily Cinchonoideae. Naucleeae has been widely accepted as a separate tribe within the subfamily Cinchonoideae. We have selected to work with two species from the Naucleeae tribe: *Sarcocephalus latifolius* and *Mitragyna rubrostipulata.*

1.5.1.1. *Sarcocephalus latifolius* (Sm.) E.A.Bruce

Nauclea latifolia is the basionym of *S. latifolius*. Both names are used in the literature about this medicinal plant. However the work of Razafimandimbison and Bremer (2002) and Bridson & Verdcourt (1988) in Flora of Tropical East Africa retain the status of *Sarcocephalus* as a separate genus and we follow their advice.

S. latifolius is a shrub or small tree growing in woodland savannas in tropical Africa (Fig. 3). The fruits are edible, but nowadays mainly used as fodder, even though it has been found to have high content of vitamin C and iron (Amoo & Lajide, 1999). Leaves, stem bark, roots and root bark are used as medicine to treat a wide range of ailments (Lye et al. 2008) like hernia, backache, uterine fibroids, diarrhoea, stomach ache, tuberculosis, gastrointestinal, helminths in man and animals, diabetes, hypertension, and urethritis (Okoli & Iroegbu, 2004). However the most common use is against malaria and effects are well documented (Benoit-Vical et al. 1998; Traore et al. 2000; Asase et al. 2005; Zirihi et al. 2005; Abbah et al. 2010). The pharmacological

activities of the plant on uncomplicated malaria are now investigated to develop an effective therapy (Abbah et al. 2010).

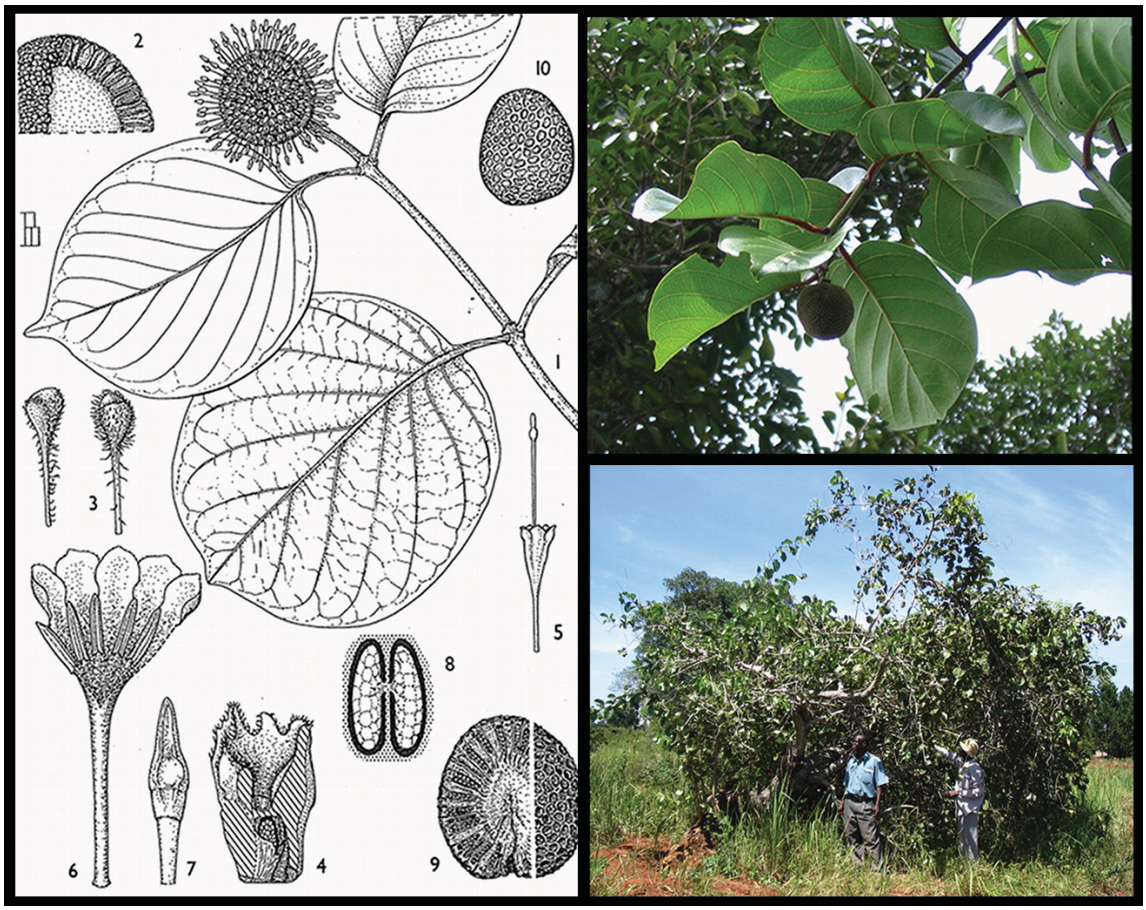

Fig. 3. Sarcocephalus latifolius, drawing from Bridson & Vercourt 1988. Right: Fruit (above) and tree harvested for roots (below). Photos: T. Stangeland

S. latifolius is one of the traditionally used plants that have undergone rather extensive phytochemical screening, which have revealed monoterpene, triterpene and indole alkaloids, (Shellard & Lala 1978; Hotellier et al. 1980; Abreu & Pereira 1998 and 2001; Ngnokam et al. 2003; Hideyuki et al. 2003), saponins (Okoli & Iroegbu 2004), sugar fractions in the bark (Abreu et al. 2001), and proanthocyanidins (Fakae et al. 2000). However extracts with high

concentration of alkaloids have also demonstrated a significant genotoxicity in human cells, and it is recommended that it should be used with caution (Traore-Keita et al. 2000). An aqueous root bark extract has demonstrated neuropharmacological effect on rodents, indicating psychoactive substances (Amos et al. 2005). A recent similar study of the same extract suggests the presence of biologically active compounds with anti-nociceptive, anti-inflammatory and anti-pyretic activities that justify the use to treat malaria (Abbah et al. 2010). Since bioactivity and chemistry is so well documented for *S. latifolius* and the fact that it is becoming locally scarce (Tabuti 2007, Okello & Ssegawa 2007), our research focuses on germination and growing experiments for this species.

1.5.1.2. *Mitragyna rubrostipulata* (Schum.) Hav

We have in our recent papers (Stangeland et al. 2007, Stangeland et al. 2010) applied the name *Hallea* for one of our major plants studied (*Hallea rubrostipulata* J.-F. Leroy). However, recently this name was found to be a homonym of *Hallea* G. B. Mathews (Deng 2007). The genus *Hallea* J.-F. Leroy can therefore not be used, and Deng (2007) replaced it with a new name, viz. *Fleroya* Y.F. Deng. However Razafimandimbison and Bremer (2002) had some years earlier re-included *Hallea* J.-F. Leroy in *Mitragyna* Korth. This latter treatment is probably the most acceptable one.

M. rubrostipulata is a tree up to 25 m high, indigenous to East Africa (Fig. 4). It grows in wet forests from Rwanda to Ethiopia and further south to Malawi, and is common in swamp forests along Lake Victoria. In the Sango Bay area in Uganda the bark of the tree is one of the most commonly used drugs to treat malaria.

During the last decades little attention has been paid on the use and bioactivity of *M. rubrostipulata,* except for one study on anti-plasmodial activity in Rwanda (Muganga et al. 2010), and a few studies on its use in Uganda (Ssegawa & Kasenene 2007a; Kamatenesi & Oryem-Origa 2005). However from the beginning of the 1900 century the genus *Mitragyna* gained considerable attention after Ridley in 1897 reported the leaves and bark of *Mitragyna*

Fig. 4. Mitragyna (Hallea) rubrostipulata, drawing from Bridson & Vercourt 1988. Right: Flowers (above) and stem harvested for bark (below). Photos: T. Stangeland

speciosa Korth. as a cure for opium addiction. The leaves of *M. speciosa* ("Kratum") were chewed for many years in Thailand as an opium substitute, but are now forbidden. The first alkaloid was isolated from *M. speciosa* by Hooper in 1907 and was later called mitragynine (Shellard 1974). Mitragynine was later found to have pain-threshold elevating and antitussative properties comparable to codeine, and no addictive properties (Macko et al. 1972). Another alkaloid found in *M. speciosa,* viz. 7-hydroxymitragynine was found to have a more potent antinociceptive activity than morphine (Matsumoto et al. 2004). Both antinociceptive and anti-inflammatory activity of methanol extract was demonstrated on rodents (Shaik Mossadeq et al. 2009). Presently the use of Kratum has gained considerable interest and increased use as self treatment of Opioid withdrawal (Boyer et al. 2007).

In West Africa *Mitragyna inermis* (Willd.) K.Schum. is commonly used to treat malaria. Traore-Keita et al. (2000) demonstrated that total alkaloids obtained by chloroform extraction had potent antiproliferate action (counteracting the process of cell division) on two *P. falciparum* strains, while aqueous extract did not show significant activity, which indicate that the pharmacological activity of *Rubiaceae* are mostly due to alkaloids.

The first alkaloid from the bark of *M. rubrostipulata:* mitraphylline was isolated by Michiels & Leroux in 1925 but the structure was not determined until 1958 by Seaton et al. (1958). Later Shellard (1978) isolated the alkaloids hirsutine, hirsuteine, rhynchophylline, isorhynchophylline, rotundifoline, isomitraphylline, rhynchophylline N-oxide and anti rotundifoline in addition to mitraphylline. All alkaloids were present in the root bark, but hirsutine and hirsuteine were not found in the leaves and stem bark. In Uganda the stem bark of *M. rubrostipulata* is used against malaria (Ssegawa &Kasenene 2007a) and to treat erectile dysfunction (Kamatenesi-Mugisha & Oryem-Origa 2005). We have not found any reports on in vitro or in vivo tests on activity, and if effect can be demonstrated, we do not know what kind of compounds cause the effect. However cat's claw (*Uncaria tomentosa* DC.) is a very popular and widely used medicinal plant from the Amazon used as an immunomodelatory, anti-inflammatory and anti-cancer remedy (Pilarski et al. 2007). *U. tomentosa* belongs to the same subfamily as the tribes Sarcocephalus and Mitragyna: Cinchonoideae. Two of the active ingredients are common with *M. rubrostipulata:* mitraphylline and isomitraphylline. Currently the standardisation of commercial formulation of cat's claw is based on the alkaloid content, but still there is some controversy whether the effect is caused by the alkaloids. Sandoval et al. (2002) argue that anti-inflammatory and antioxidant effect must be caused by some other active principle than alkaloids, while Garcia Prado et al. (2007) demonstrated promising antiproliferative effect of mitraphylline on human glioma and neuroblastoma cell lines, and Pilarski et al. (2007) indicate that isomitraphylline and pteropodine are the most suitable substances for standardisation of cat's claw preparations. It is well known that the alkaloid content show large variation according to time of year of harvesting and growing conditions for different *Mitragyna* species (Shellard 1974) as well as for *Uncaria* (Pilarski et al. 2007).

1.5.2. Asteraceae

The sunflower family is the largest family of flowering plants with about 1315 genera and 21000 species; in East Africa 137 genera and 800 species (Lye et al. 2008). Chemical characteristics in the family are presence of sesquiterpene lactones, polyacetylenic compounds (polyenes) and essential oils. Some taxa accumulate pyrrolizidine alkaloids, which may be toxic to the liver (Heinrich et al. 2004). The most important new drug to treat malaria is extracted from *Artemisia annua* in this family. In Africa many plants from Asteraceae are used to treat malaria. The most common and well documented for effect is *Vernonia amygdalina* Delile (Challand & Willcox 2009). Other species less documented are *V. lasiopus* O.Hoffm. and *V. adoensis, Aspilia africana* (Pers.) C.D.Adams*, A. pluriseta* Schweinf. ex Engl., *Bidens pilosa* L.*, Conyza bonariensis* (L.) Cronquist*, Microglossa pyrifolia* Kuntze and *Tithonia diversifolia* A.Gray (Lye et al. 2008).

1.5.2.1. *Vernonia adoensis* Sch. Bip. ex Walp

Fig. 5. Vernonia adoensis, drawing from Bentje 2000. Flowering herb (right). Photo: Ingvild Austarheim

The Vernonieae is one of the major tribes in Asteraceae with more than 1500 taxa. It has been called the “evil” tribe because overlapping character states make taxonomic delimitations difficult (Keeley et al. 2007). In literature the name *Vernonia kotschyana* is commonly used, but according to Beentje (2000) this is a synonym or a subspecies of *V. adoensis.* Keeley et al. (2007) showed that *Vernonia adoensis* does not belong to the typical genus *Vernonia.* They placed the species in the genus *Baccharoides,* but until a full molecular treatment of the African *Vernonia* has been undertaken we have chosen to use the name given by the “Flora of Tropical East Africa” (Beentje 2000).

Vernonia adoensis is an herb or small shrub growing in savanna from Senegal to Nigeria, across Africa to Ethiopia and in South Africa (Fig. 5). It is widely used in African folk medicine to treat diarrhoea, dizziness, vomiting, impotence, abdominal pain during menstruation, gonorrhoea, tuberculosis (Lye et al. 2008) , gastrointestinal disorders and wound healing (Nergard et al. 2004).

Based on the use of *V. adoensis* as a remedy for stomach ache in Nigeria, Deeni & Hussain (1994) did a study of antimicrobial activity of the root extracts. They revealed antimicrobial activity against Grampositive and Gramnegative bacteria, and found that some plant extract tested positive for alkaloids.

Minute amounts of two glaucolides were isolated from the aerial parts of the plant (Bohlmann et al. 1984). Glaucolides are highly oxiginated sesquiterpene lactones common in the genus *Vernonia,* but were earlier believed to be found mainly in the New World species. Sesquiterpenes are constituents in many volatile oils, like chamazulene and bisabolol in chamomile and absinthin in *Artemisia absinthium* L.. Artemisinin, one of the active compounds extracted from *Artemisia annua,* is a sesquiterpene as well. Sanogo et al. (1998) isolated five new stigmastane steroidal glycosides and a new androst-8-en glycoside from the root of *V. adoensis,* and Nergard et al. (2004) isolated and did a partial characterisation of immunomodulating polysaccharides from the roots. The dried and powdered root have now been tested and developed to ‘Gostrosedal’, one of the Improved Traditional Medicines (ITM) registered in Mali for the treatment of gastric ulcer (Nergard 2004).

A screening for antifungal, larvicidal, molluscicidal, antioxidant and radical scavenger activity in four Malian medicinal plants were performed (Diallo et al. 2001). The methanol (MeOH) extract from the roots and the dichloromethane (DCM) extract of the leaves of *V. adoensis* showed both antioxidant (β-carotene) and radical scavenging (DPPH) activities. Larvicidal activities were observed in the DCM extract of the leaves against *Culex quinquefaciatus* (transferring lymphatic filariasis) and *Anopheles gambia* (transferreing *Plasmodium falsiparum*), while the MeOH extract from the leaves also exhibited molluscicidal activity against *Biomphalaria pfeifferi* and *B. truncates* (both possible vectors for schistosomiasis).

1.5.3. Rutaceae

Rutaceae is a family of about 150 genera and 900 species, widely distributed in tropical and subtropical regions and with 14 genera and 42 species in East Africa (Lye et al. 2008). Several genera have species with medicinal use like *Ruta, Toddalia, Zanthoxylum* and the important fruit genus *Citrus.* Essential oil is common in many taxa, as well as alkaloids, especially benzyltetrahydroisoquinoline, acridone and imidazole types in addition to furanocumarins and pyranocumarins (Heinrich et al. 2004).

1.5.3.1. *Zanthoxylum chalybeum* Engl.

Fagara chalybea (Engl.) Engl. is a synonym for *Z. chalybeum,* also called East African prickly ash (Fig. 6). It is a spiny deciduous shrub or tree up to 8 m tall growing in medium to low altitudes in dry woodland or grassland, often on termite mounds, from Ethiopia to South Africa. The bole has characteristic conical woody knobs with sharp prickles, the leaves are compound, with 6-9 shiny leaflets with a strong lemon scent if crushed, and hooked prickles on the leaf stalk. Leaves, bark and roots are used for medicine (Katende et al. 1995). An infusion or decoction of the bark is widely used for many ailments like colds, coughs, fever, stomach complaints, oedema, epilepsy and sleeping sickness (Lye et al. 2008). Decoction of the roots are used to treat infertility and uterine fibroids in Uganda, rheumatism in Kenya, hook worms in Tanzania; while cold water extract of fresh roots is drunk against bilharzias. The most common use in many countries is against malaria, and Gessler et al. (1994) found *Z. chalybeum* (root

bark) to be one of the four most efficient antimalarial plants of 43 species tested. Hamza et al. (2006) found the MeOH extract of the root bark to be one of the nine best extracts among 56 species with the broadest antifungal activity (effect towards at least four different yeasts). In a screening for in vitro antibacterial and anti-inflammatory activity Matu & van Staden (2003) found the MeOH extract of the root bark to be one of the five best among 12 species with the highest activity towards three Gram-positive bacteria. The stem bark had good activity against one of the bacteria. Kuglerova et al. (2008) found high antioxidant potential in crude ethanol extract of stem bark, while Rukunga et al. (2009) found the water extract of the root bark to be the most active anti-plasmodial among 12 plants used against malaria in Kenya. In a similar study from Rwanda MeOH and dichloromethane extracts of *Z. chalybeum* root bark, were one of the five most active anti-plasmodial among 13 plants tested (Muganga et al. 2010), while the stem bark had weak activity.

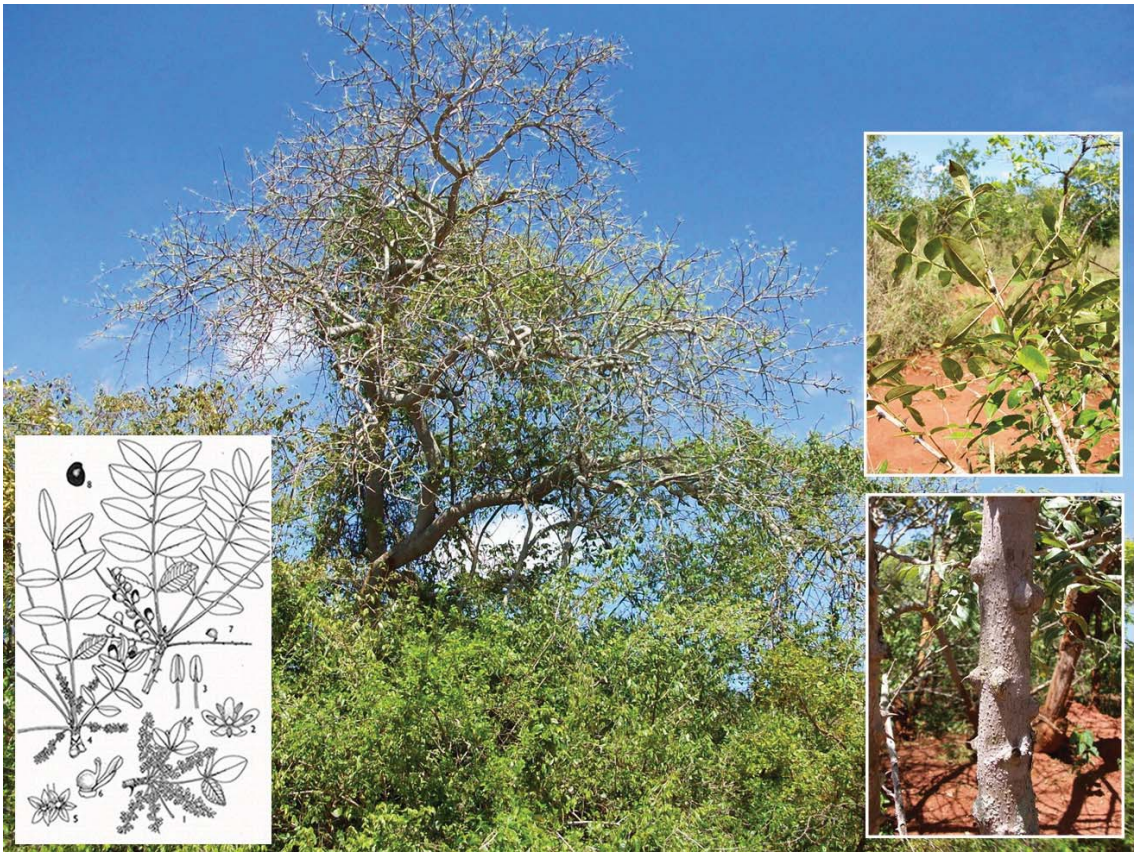

Fig. 6. Zanthoxylum chalybeum. Drawing from Kokwaro 1982. Photos: T. Stangeland

Several alkaloids have been isolated from stem and root of *Z. chalybeum.* Taniguchi et al. (1985) isolated two 2-quinoline alkaloids, flindersine and *N*-methylflindersine as insect anti-feedants, and found them to be general growth inhibitors or biological poisons. Nakatani et al. (1990) isolated the isoquinoline alkaloid jatrorrhizine chloride as an antimicrobial component from the root bark, along with hesperidine, lupenone, lupeol, (-)-asarinin and 2-tridecanone. According to Fish & Waterman (1972), candisine, phenylethylamine, benzylquinoline (tembetartine) and benzophenanthridine (chelerythrine, nitidine) alkaloids are produced in highest quantity in *Z. chalybeum* bark, while Kamikawa et al. (1996) reported broad anti-microbial activity of three 2-quinolone alkaloids: flindersine, *N*-methylflindersine and 7,8-dimethoxy- *N*-methylflindersine. However Kato et al. (1996) found tembetarine, jatrorrhizine, (-)-cis-N-methylcanadine, nitidine and chelerytrhine to be the main alkaloids, and protoberberine to be present as well. What compound causes reported anti-plasmodial effect is yet to be established.

In another resent paper from Kenya, an inventory of plants commonly used for the treatment of malaria was done to get a basis for selection of plants for further pharmacological, toxicological and phytochemical studies (Nguta et al. 2010). *Z. chalybeum* was one of the three most commonly mentioned plants used. However the authors warn that the plants in the study mainly were collected from community land, which is facing great pressure due to overutilization of indigenous trees and hence they may disappear before they get investigated. We had the same experience in Kaliro district. We went for two full days' excursions to find *Z. chalybeum* trees, with local healers joining us. We only found root suckers from cut down trees. We were told that it was cut down for char coal production, since the wood make high quality charcoal, and this is one of few ways to earn money in the district. We had to go about 50 km north of Kampala to find mature *Z. chalybeum* trees that could be harvested for bark. In our nursery and planting experiment we did not manage to get hold of seeds, but tried to make seedlings from root suckers, but without success.

2. OBJECTIVES

The main purpose of this study is to contribute to improved health for people and environment in some rural areas in Uganda through the use of local knowledge and resources, examined by modern tools.

The specific objectives addressed in this thesis are:

1. Discover the situation for Traditional Medicine and legislation concerning conservation and use of biodiversity in East Africa with Tanzania as an example (Paper I).

2. Examine germination and seedling growth of, and test the frame work species method as a useful tool to conserve medicinal trees and the environment (Paper II and III).

3. Determine the antioxidant activity in commonly available fruits and vegetables in Uganda. Is the antioxidant activity in traditional diets sufficiently high to prevent oxidative stress and thus combat disease? (Paper IV)

4. Investigate the anti-plasmodial and antioxidant activity in raw extracts from three medicinal plants commonly used against malaria in different parts of Uganda: *Mitragyna rubrostipulata, Vernonia adoensis* and *Zanthoxylum chalybeum* and if there is a correlation between the different bioassays (Paper V).

5. Document how traditional healers and birth attendants use plants to treat malaria in Mbarara district, Uganda (Paper VI).

3. MATERIAL AND METHODS

3.1. Study areas

Paper I is mainly a review paper, but some fieldwork (interviews) was done in Northern Tanzania. Fieldwork for paper II was done in Kaliro district (1 in Fig. 7) and Sango Bay (2 in Fig. 7) area in Uganda, while for paper III it was conducted in Kaliro district. The food analysed in paper IV was collected or bought at local markets in Uganda, while the medicinal plants analysed in paper V were collected from Sango Bay, Mbarara and Nakosongola District. Interviews for paper VI was performed in Mbarara district (3 in Fig. 7). I was introduced in the field areas by Ugandan colleagues, who had previously done their PhD work in the same areas. Prior to any contact with local people, the study and its objectives were introduced to the local authorities and permission to do research in the area requested for.

Fig. 7. Map of Uganda, with the three main areas of field work: 1. Kaliro District, 2. Sango Bay area, 3. Mbarara District http://www.lib.utexas.edu/maps/cia10/uganda_sm_2010.gif

3.1.1. Kaliro District

Kaliro District is situated 200 km northeast of Kampala, 33º30´-33 º35´ E and 1º04´-1º15´ N at an elevation of 1030-1080 m above sea level. The district has two rainy seasons, March-May and August-September. The mean annual maximum temperature is 30-32.5ºC and mean annual rainfall is 1250-1300 mm (Anonymous 1967). The district is heavily inhabited with a population density in excess of 280 people per square kilometer. Much of the original vegetation has been destroyed and the landscape converted to small scale farmland. The district is dominated by small scale farms and papyrus swamps (Fig. 8).

Fig. 8. View from Muli hill, Gadumire, Kaliro district, towards Lake Kyoga. Photo: T. Stangeland

3.1.2. Sango Bay area

The Sango Bay Forest Reserve is located in Rakai District in Southern Uganda near Lake Victoria and near the border to Tanzania (0º47´- 1 º 00´S and 31º28´ – 31º43´E). The Reserve has a mean annual maximum temperature of 25-27.5° C and mean annual rainfall of 1300-1500 mm (Anonymous 1967). 1/3 of the forest reserve is dense swamp forest, while 2/3 is grassland. The inhabitants are mainly subsistence farmers living in small scattered settlements (Fig. 9).

Fig. 9. View from a hill towards the Sango Bay Forest reserve.
Photo: T. Stangeland

3.1.3. Mbarara District

Mbarara District is located in Western Uganda 270 km southwest of Kampala. The fieldwork was mainly done in Nyakayojo subcounty (0º63´S and 30.61ºE) (Paper VI). The area has a mean annual maximum temperature of 25-27.5 °C, and mean annual rainfall of 900-1000 mm (Anonymous 1967). The area is hilly and the main economic activity is mixed farming, cultivation of crops and grazing cattle/ goats (Fig. 10).

Fig. 10. View from a hill towards the south and the hills of Nyakayojo.
Photo: T. Stangeland

3.2.

Ecology and conservation of medicinal plants

3.2.1. Phenology

The selection of species to study was based on previous studies (Ssegawa & Kasenene, 2007a,b; Tabuti (2007); Tabuti et al. (2009), and a group discussion with Traditional Healers in Gadumire.

Phenology studies were performed on *Mitragyna (Hallea) rubrostipulata, Syzygium guineense* DC. and *Warburgia salutaris* (G.Bertol.) Chiov. in Sango Bay forest reserve in 2006 and 2007, and on *Capparis tomentosa* Lam.*, Psorospermum febrifugum, Sarcocephalus latifolius* and *Securidaca longepedunculata* Fresen. in Kaliro District from November 2006 to November 2007 (Paper III). Leafing, flowering and fruiting development were recorded every second week on 3 to 30 individuals of each species. The low number of some species was due to the rarity of plants in the area of study.

3.2.2. Germination

Little is known (published) about germination and growth conditions of indigenous trees in East Africa. Our germination experiments were done both in field, laboratory (Paper II) and in nursery (Paper III). In the field experiment we tested germination ability of *Sarcocephalus latifolius, Securidaca longipedunculata* and *Capparis tomentosa* in different degrees of soil disturbance and light regime. *S. latifolius* failed to germinate, *S. longipedunculata* germinated but the seedlings died after some time and the seeds of *C. tomentosa* turned out to be too old and not viable. The experiment was repeated in November 2005 using the same plots but with different treatments, but without success. Since the field germination experiment did not succeed, we decided to do experiment in laboratory.

Seeds of *S. latifolius* and *Mitragyna (Hallea) rubrostipulata* were tested in a laboratory germination study which lasted for four weeks (Paper II). The seeds were incubated in five controlled environment cabinets at constant temperatures of 15, 20, 25, 30 and 35 °C under 12:12 h light/dark.

The nursery germination experiment lasted for one year, and 27 species of mainly indigenous medicinal trees, but also some introduced trees classified as fruit or non timber forest trees, were tested and germination registered every 3rd day (Paper III).

3.2.3. Seedling growth

The laboratory study of seedling growth lasted for 12 weeks (Paper II). Seeds were sown on saturated filter paper in 9 cm Petri dishes and placed in a controlled environment cabinet at 25 °C. After germination the seedlings were transferred to pots (8 cm) and placed in five growth chambers at constant temperatures of 15, 20, 25, 30 and 35° C under 12:12 h light/dark. Five seedlings of each species were harvested, dried and weighed at the time of planting (time 0). After week four plants from each temperature were harvested, separated into shoots and roots, dried at 70 °C for 48 hours and then weighed. Harvesting continued every second week until the 12th week after planting.

3.2.4. Framework species method

We wanted to test the propagation and growing abilities of medicinal trees in the field, and we chose to use a slightly modified Framework species (FWS) method (Paper III). The method is developed to restore degraded tropical forest, but we wanted to test it as a method to conserve medicinal trees, make a kind of multipurpose tree gardens as a measure to prevent unsustainable harvesting of locally rare species, and to provide the traditional healers with raw material for their practice and other woody products. The selection of species was based on a group discussion with the traditional healers in Gadumire at the beginning of the study. They were asked to name five medicinal plants that they regarded as the most important in their work, and that also were getting difficult to find. They came up with five woody species: *Capparis tomentosa, Securidaca longipedunculata, Maytenus senegalensis* (Lam.) Exell*, Sarcocephalus latifolius* and *Psorospermum febrifugum.* Other species were selected based on a study on community preferences on woody species by Tabuti et al. (2009). Since we added just a little

cow manure at planting, we decided to include two agroforestry species that fix nitrogen and can be used for mulching and fodder (*Calliandra calothyrsus* Meisn. and *Leucaena leucocephala* (Lam.) deWit). The *Senna* species were included mainly to provide firewood.

In the study we specifically determined the phenology (for four of the species), germination behavior, survival and growth after the planting of 27 mainly indigenous woody species. We tested for germination traits as described by Blakesley et al. (2002), raised seedlings for 4 to 12 months, planted a mixture of 27 species of mainly medicinal trees at a density of 3125 ha^{-1} in three plots in Kaliro district and monitored them for survival and growth for thirteen months broadly as described by Elliott et al. (2003).

3.3. Bioactivity in food and medicinal plants

3.3.1. Antioxidant activity

Three different methods were used to analyse for antioxidant activity. The Ferric Reducing Ability of Plasma (FRAP) were used to analyse fruits and vegetables (Paper IV). This work stretched over several years, and since we started using only this method, we decided to finish the study in the same way in order to have comparable results.

In addition to the FRAP assay the DPPH radical scavenger method and total phenol content using the Folin-Ciocalteu reagent was used to test medicinal plants for antioxidant activity (Paper V).

3.3.2. Extraction of plant material

Powdered bark of *Mitragyna (Hallea) rubrostipulata* stem bark*, Vernonia adoensis* leaves and *Zanthoxylum chalybeum* stem bark was extracted with five solvents: acetone, dichloromethane, methanol, a mixture of chlorophorm, methanol and water, and water. The solutions were centrifuged at 1800 x g for 5 min, and this was repeated 3 times, mainly as described by Elloff (1998), then concentrated to dryness and stored at -20 °C until use.

3.3.3. Anti-plasmodial activity

Anti-plasmodium effect was tested using an enzyme-linked assay (ELISA) that quantifies the parasite histidine–rich protein-2 (HRP2). This protein is produced by the *Plasmodium falciparum* parasite, it is closely related to the development and proliferation of the parasite and therefore is perfectly suited to reflect growth inhibition as a measure of drug susceptibility (Noedl et al. 2002).

3.3.4. Methods to detect groups of compounds in raw extracts

3.3.4.1. Thin layer chromatography (TLC)

TLC is a method used to separate compounds in mixtures, in this case the raw extracts. We used aluminium sheets coated with silica (stationary phase). In this method a small spot of each raw extract is applied to the plate about 1 cm from the bottom, then the plate is placed in a sealed container holding a suitable solvent or solvent mixture (the mobile phase). The mobile phase is drawn up the plate by capillary action, meets the sample mixture and reacts with it. The different compounds in the sample mixture will then travel at different rate due to different affinity to the stationary phase, and because of differences in solubility of the compounds. We also used spray reagents to better see the separated compounds, in visual light or under UV. We followed the standard procedures described by Wagner and Blatt (1996) for selection of mobile phase and spray reagents for detection of the main groups of secondary compounds that we expect to discover in the raw extracts, namely: alkaloids, flavonoids, saponins and bitter principles.

3.3.4.2. Nuclear Magnetic Resonance (NMR)

NMR is a method to detect the structure of a chemical compound. Used on a mixture, like our raw extracts, it can tell something about what kind of compounds may be present and which are not.

3.4. Ethnopharmacological survey of plants used to treat malaria

The ethnopharmacological survey was carried out in March and April 2009 in Nyakayojo subcounty in Western Uganda. Semistructured interviews using a questionnaire were conducted with 28 traditional healers and traditional birth attendants from the 'Nyakayojo Traditional Healers and Traditional Birth Attendants Association', which has members from the whole subcounty. We had previously been working with this group in two workshops. A group of three persons were performing the interviews. The other two were Esther Katuura, who comes from the area, speak the local language fluently and did her PhD study in Pharmacology on plants used to treat malaria, and Paul Alele who is a physician working at Mbarara University. The healer's informed consent was sought before performing the interviews.

4. RESULTS AND DISCUSSION

4.1. Recognition and development of traditional medicine in East Africa

The aim of Paper 1 was to trace developments in traditional medicine (TM) and legislations concerning conservation and use of biodiversity in Africa with Tanzania as an example. The study showed that TM has been progressively paid attention to and is included in the development plans of Tanzania. The Government has decided to encourage the integration of TM into the primary health system. In 2002 The Parliament passed *The Traditional and Alternative Medicine Act,* which became operational in 2005 with the aims of integrating TM in primary health care and encouraging cooperation between TMPs and western trained doctors.

Two of the botanically most important ecological zones in Tanzania; Coastal forests and Mountain forests, suffer from severe degradation, 70-90% of forests outside Protected Areas being destroyed. Much of the medicinal plants are found in these zones. In new regulations and laws, the aim is to use the genetic resources for the benefit of present and future generations. The main legal framework seems to be in place, the Forest laws that also contain regulations on prior informed consent, access and benefit sharing are now operational.

The world market in MAPs is estimated around US $60 billions, and in countries like China and India this trade is becoming increasingly important. In Africa there is a considerable informal

trade in MAPs from TMs and in markets, but it is difficult to estimate the value of this 'hidden economy'. Experience from China, India and South Africa shows that sustainable harvesting is not sufficient to save threatened species, and in China and South Africa there are initiatives to cultivate MAPs. It is important to intensify studies on populations and sustainability of harvesting of medicinal plants in Tanzania. Sustainable harvesting and growing of medicinal plants have the potential of accelerating rural development. Some local stakeholders have already started a process that can contribute to this development. Pharmacological studies to confirm safety and effectiveness of medicinal plants are now being done, but still very few plants have been screened.

4.2. Ecology and conservation of food and medicinal plants

4.2.1. Germination and early seedling growth experiment

The aim of Paper II was to investigate the influence of light and temperature on the establishment of two Ugandan medicinal trees; *Mitragyna rubrostipulata (Hallea rubrostipulata),* and *Sarcocephalus latifolius*. The trees are regarded as important in the local health system, are getting scarce in the areas of study and turned out to be problematic to grow in a field setting. In Gadumire, Kaliro we tried to sow in an experimental field, but did not succeed. In Sango Bay a local nursery project tried to germinate *M. rubrostipulata* without success.

Seeds from the trees were collected in these two areas, dried and brought to Norway. We set up two experiments, one on germination conditions, and one on early seedling growth. Our main findings were that both species needed light to germinate; *Mitragyna rubrostipulata* had a temperature optimum of 25 °C with 79% germination, while the total germination after 28 days was close to 60% for temperatures between 20 and 35 °C for *Sarcocephalus latifolius.* Seedlings of *M. rubrostipulata* died at 35 °C, and seedlings of *S. latifolius* died at the low temperature of 15 °C (Fig. 1 and 2, Paper II). The temperature preferences of the two species reflect the temperature of their natural habitat. Both seeds and seedlings are very small for both species. This may be one of the reasons why the seedlings had difficulties to establish. In the Sango Bay forest we could not see rejuvenation of *M. rubrostipulata,* and in the beginning the nursery group

had problems germinating the seeds. It is possible that they covered the seeds with too much soil for them to germinate. In Kaliro reasons for failure may be predation or that the soil in the degraded area where we did the growing experiment was not able to retain water long enough for the seedlings to establish.

For both species, the seedlings had their best growth at 30 °C. *Sarcocephalus latifolius* had a much more rapid growth in the period of study. After 12 weeks, dry weight of shoots and roots of *S. latifolius* was almost 10 times higher than that of *M. rubrostipulata* (Fig. 3 and 4, Paper II). However, we found a striking difference in the allocation of biomass to roots and shoots in the two species. While at the end of the experiment *M. rubrostipulata* had almost as much biomass in roots as in shoots, the allocation of biomass to shoots for *S. latifolius* was almost twice as high as to roots. This may be an adaptation by the *Mitragyna* seedlings to the oligotrophic environment they grow in.

We concluded that nursery assistance is needed to establish healthy populations of the two species of study and probably many other endangered species.

4.2.2. Phenology and cultivation of some selected woody species

The aim of this study was to (1) gain knowledge about flowering and fruiting phenology and seed germination traits of selected medicinal trees, (2) to assess each species potential as a framework species, and (3) to find out if this method is suitable to conserve medicinal trees and the environment in Kaliro district, Uganda (Paper III).

4.2.2.1. Phenology

Phenology was performed in two areas: in Sango Bay the species *Mitragyna (Hallea) rubrostipulata, Warburgia salutaris* and *Syzygium guinence,* and in Kaliro District *Sarcocephalus latifolius, Securidaca longipedunculata, Capparis tomentosa* and *Psorospermum febrifugum* was monitored for about one year (only data from the species that were included in the planting project is presented in the paper).

We found that between December 2005 and December 2006 there were 2 fruiting periods for *Mitragyna rubrostipulata,* with peak flowering in January to February and a smaller flowering in May to June. Peak fruiting was in March and September. The flowering was in the dry season, and peak fruiting as the rain started. For *Warburgia salutaris* peak fruiting was recorded in September 2006 and March 2007, and for *Syzygium guinense* peak fruiting was found to be in July 2006.

In Kaliro we observed mature fruits on *Sarcocephalus latifolius, Securidaca longipedunculata* when we started registration in December 2006. For *S. latifolius* a new fruiting period started in February 2007, but the fruits took very long to mature, and peak fruiting was not until July/ August. *S. longipedunculata* had a new flowering period in April, but almost all flowers were destroyed by a hailstorm. *P. febrifugum* had long flowering (March-July) and fruiting (April-November) period, possibly because the birds ate the fruits as soon as they ripened. This fact also made it difficult for us to make seedlings from this species, as it was almost impossible to get ripe fruits. For *Capparis tomentosa* no flowers or fruits were recorded during the time of study.

4.2.2.2. The Framework species method

Field performance was assessed by monitoring survival, height and crown width once every month for 13 months after planting. Eleven species (*Artocarpus heterophyllus, Calliandra calothyrsus, Callistemon citrinus* Skeels, *Carica papaya, Carissa spinarum* L.*, Leucaena leucocephala, Markhamia lutea* K.Shum.*, Sarcocephalus latifolius, Senna siamea* (Lamarck) H.S.Irwin & Barneby*, S. spectabilis* (DC.) H.S.Irwin & Barneby and *Terminalia schimperiana* Hochst. ex Delile) proved to be excellent framework species. Eight species qualified as 'acceptable' FWS (*Albizia coriaria* Welw.*, Ceiba pentandra, Entada abyssinica* Steud.*, Erythrina abyssinica* Lam.*, Eugenia jambos* L.*, Ficus sycomorus* L.*, Maesopsis eminii* Engl. and *Milicia excelsa* (Welw.) C.C.Berg*),*while seven species were ranked as 'marginally acceptable' (*Acacia macrothyrsa* Harms*, Calpurnia aurea* Benth.*, Canarium schweinfurthii* Engl.*, Capparis tomentosa, Ficus natalensis* Hochst.*, Senna sp.* and *Warburgia salutaris). Annona squamosa* Vell. was the only species rejected since both germination and survival was low. Whether the

content of active compounds are as high in the cultivated, as in the wild growing plants, still need to be investigated. We believe that if they are planted in a mixed stand with mainly local trees, and without too much input of nutrients and water, it is likely that the content of active principles are close to the plant harvested in the wild.

We succeeded to cultivate two of five important medicinal woody species named by the traditional healers at the beginning of the study: *Sarcocephalus latifolius* and *Capparis tomentosa. Securidaca longipedunculata* we managed to germinate, but when the seedlings reached a certain size between 10 and 20 cm they started to wither. A few plants were planted in the plots, but none survived until the last monitoring. For *Psorospermum febrifugum* we did not manage to get ripe seeds because the birds were eating them, and for *Maytenus senegalensis* there were so few shrubs left in the area that we did not manage to get seeds at all. *Zanthoxylum chalybeum* is in great need of conservation measures, but this species also need further study.

Trees with good reaforestation traits could be recommended for planting while the species that were marginally acceptable or rejected require extra research since some of them are important medicinal woody species of conservation concern.

4.3. Antioxidants in fruits and vegetables

The results show a great variation in antioxidant activity (AOA) ranging from 72.3 ± 13.5 (*Syzygium cumini* (L.)Skeels seed) to 0.09 ± 0.05 (pumpkin fruit) mmol /100g fresh weight (FW). We estimate serving sizes and determine the total dietary antioxidant (TDAC) per day of three traditional Ugandan diets. The dietary plants with highest AA per serving size are pomegranate, *Canarium schweinfurthii,* guava, mango and tree tomato with values ranging from 8.91 to 3.00 mmol/serving. Of the traditional diets the central/eastern (C/E) and the western (W) diets have almost the same AOA (9.31-9.78 and 9.75 mmol/day), while the northern (N) diet has an AOA of 7.50-8.02 mmol/day. In another dietary study from Norway average intake of antioxidants was 17 mmol/day. In addition many Ugandans do not eat the full diet of 3 meals per day, so in reality, the intake of antioxidants are lower. Some of the fruits and vegetables that are high in antioxidants, like mango, papaya and the green leafy vegetables are also rich in vitamin

A and/ or iron. These are main nutrient deficiency in East Africa, and intake of these food items should be encouraged.

4.4. Bioactivity in extracts from three plants used to treat malaria

During malaria infection increased reactive oxygen species are generated that may contribute to erythrocytic damage and anaemia. On the other hand some hypothesise that important antimalarials act as pro oxidants in the plasmodium parasite. In any case oxidative stress seems to play an important role in erythrocytes and parasites during malaria infection. In our bioassays several plant extracts showed high anti-plasmodial as well as high antioxidant activity. We do not know if it is the same or different compounds in the raw extracts that have these effects, and neither do we know at this stage the bioavailability of the active principles. But since the water extracts of these plants have been traditionally used against malaria there is reason to believe that there is some bioavailability. We believe that it may be favourable to select extracts with high anti-plasmodial and antioxidant activity for further investigation. In future work the most promising raw extracts, like the water extract of *Mitragyna rubrostipulata* and *Vernonia adoensis* should be tested in bioassay guided fractionation for antimalarial effect and toxicity to see if effective and safe antimalarials can be developed.

4.5. Plants used to treat malaria

We interviewed 28 traditional birth attendants and healers. Altogether they used 57 different plant species to treat malaria (Paper VI). The most frequently used plant was *Vernonia amygdalina* used by 86% of the informants. The leaves of *V. amygdalina* are widely used in many African countries (Lye et al. 2008) and the effect on uncomplicated malaria has recently been documented in a clinical trial (Challand & Willcox 2009). Other plants used by many were *Aloe* sp. (75%), *Justicia betonica* (39%), *Vernonia adoensis* (32%) and *Tithonia diversifolia* (29%). Around half of the species was only used by one of the respondents. Most treatments were administered orally, but baths and steam baths were also applied. The study showed that a

relative limited selection of informants use a wide range of plant species and recipes in the treatment of malaria.

4.6. Benefit sharing

We had meetings with our cooperating groups of healers and traditional birth attendants during every visit to Uganda. Research information was continuously communicated to the informants. The group of traditional healers in Gadumire, that we cooperated with during the longest period, was given some packing material for their herbs. One of the group members was a traditional birth attendant, and was provided with a bed for deliveries, and other equipment she needed. After the end of the growing project three groups of healers were each left with a medicinal plant garden, of around 400 trees, and each group also received a handbook on medicinal plant garden in Uganda (Adriaens 2006). Each of the healers that had taken part in the project was also provided with seedlings to plant at their homestead. For the traditional birth attendants in Mbarara, who gave interviews on their use of medicinal plants to treat malaria, we have run several workshops with Dr. Maud Kamatenesi and Rosalyne Acholi from the THETA (www.theta.org), and they have also been provided with some basic articles for their practices.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

This study shows that traditional medicine is included in National policies in both Tanzania and Uganda, and the government encourage the development of research and product development. We found that it is possible to cultivate some of the medicinal trees that are of conservation concern. Some plants need further attention. The framework species method seems to be a suitable method to provide medicinal as well as other useful tree species.

Hunger and malnutrition is still the number one threat to health worldwide, financial crisis and increased food prices have worsened the problem in recent years. In Western countries major campaigns have convinced people to eat more fruits and vegetables to keep healthy. Strangely

little emphasis has been put on the importance of food for health in developing countries. Our study shows that many easily available fruits and leafy vegetables in Uganda both are high in antioxidants and other substances, which are deficient in East African diets like iron and vitamin A. Hopefully in the future more combined efforts from researchers, authorities and donors will find out how diets could become more adequate in a sustainable way.

Malaria is a major problem, especially for pregnant women and children. The persistence of the illness is related to poverty, malnutrition, and the fact that the *Plasmodium falciparum* parasite has developed resistance toward the most common and affordable medicine. Resistance towards the new, efficient compound artemisinin has been detected in South East Asia. It is possible that it takes longer time for the parasite to develop resistance towards phytomedicines than allopathic medicines, since the content in the phytomedicines is much more complex and the preparations never completely identical. Some of the extracts analysed in this study showed good anti-plasmodial activity, and should be further analysed. It is of great importance to find new compounds to fight the parasite both to develop allopathic medicines, but not the least important: to develop efficient and safe phytomedicines. Such approaches have the potential to improve both income and health.

6. REFERENCES

Abbah J, Amos S, Chindo B, Ngazal I, Vongtau HO, Adzu B, Farida T, Odutola AA, Wambebe C, Gamaniel KS, 2010. Pharmacological evidence favouring the use of *Nauclea latifolia* in malaria ethnopharmacy: Effects against nociception, inflammation, and pyrexia in rats and mice. Journal of Ethnopharmacology 127, 85-90.

Abreu P & Pereira A, 1998. A new indole alkaloid from *Sarcocephalus latifolius.* Heterocycles 45(5), 885-891.

Abreu P & Pereira A, 2001. New aldole alkaloids from *Sarcocephalus latifolius.* Nat. Prod. Lett. 15(1), 43-48.

Abreu P, Pereira A, Relva A, 2001. Characterisation of sugar fraction from Sarcocephalus latifolius stem bark extract. Carbohydrate Polymers 45, 155-160.

Adriaens M. 2006. Family Medicinal Plant Gardens in the Rwenzori Region, SATNET, Marianum Press, Kampala.

AED (Academy for Educational Development), 2010. Africa's Health in 2010. http://www.aednutritioncenter.org/programs/africas-health-in-2010 , accessed May 25th 2010.

Amoo IA, Lajide L, 1999. Chemical composition and nutritive significanceof the fruits of Nauclea latifolia. Revista Italiana delle Sostanze Grasse, 76, 331-332.

Amos S, Abbah J, Chindo B, Edmond I, Binda L, Adzu B, Buhari S, Odutola AA, Wambebe C, Gamaniel K, 2005. Neuropharmacological effect of the aqueous extract of Nauclea latifolia root bark in rats and mice. J Ethnopharmacol 97, 53-57.

Anonymous, 1967. Atlas of Uganda. Department of Lands and Survey, Entebbe, Uganda.

Asase A, Oteng-Yeboah, AA, Odamtten GT, Simmonds MSJ, 2005. Ethnobotanical study of some Ghanian anti-malarial plants. Journal of Ethnopharmacology 99, 273-279.

Benoit-Vical F, Valentin A, Cournac V, Pelissier Y, Mallie M, Bastide J-M, 1998. In vitro antiplasmodial activity of stem and root exstracts of Nauclea latifolia S.M. (Rubiaceae). Journal of Ethnopharmacology 61, 173-178.

Bentje HJ, 2000. Flora of Tropical East Africa, Compositae Part 1, Ed. HJ Beentje HJ, Smith SAL, A.A. Balkema/Rotterdam/Brookfield.

Benzie IFF, Strain JJ, 1996. The ferric reducing ability of plasma (FRAP) as a measure, Anal. Biochem. 239, 70-76.

Blakesley D, Elliot S, Kuarak C, Navakitbumrung P, Zangkum S, Anusarnsunthorn V, 2002. Propagating framework tree species to restore seasonally dry tropical forest: implications if seasonal seed dispersal and dormancy. For Ecol Manage 164,31-38

Blomhoff R, Carlsen, MH, Andersen LF, Jacobs DR Jr., 2006. Health benefits of nuts: potential role of antioxidants. British Journal of Nutrition, 96, S52–S60.

Bohlmann F, Scheidges C, Misra LN, Jakup J, 1984. Further glaukolides from South African Vernonia species. Phytochemistry 23, 1795-1798.

Boyer WB, Babu KM, Macalino GE, Compton W, 2007. Self-Treatment of Opioid Withdrawal with a dietary Supplement, Kratom. American Journal of Addictions 16, 352-356.

Bremer B, 1996. Phylogenetic studies within the Rubiaceae and relationship to other families based on molecular data. Opera Bot. Belg. 7, 33-50.

Bridson D, Verdcourt B, 1988. Flora of Tropical East Africa. Rubiaceae. AA Balkema/Rotterdam/Brookfield, pp. 439-441.

Caulfield LE, Richard SA, Black RE, 2004. Undernutrition as an underlaying cause of malaria morbidity and mortality in children less than five years old. Am. J. Trop. Med. hyg. 71(2), 55-63.

CBD, 2010. The Convention on Biological Diversity. http://www.cbd.int/convention/ accessed June 9th 2010.

Center for Disease Control and Prevention, 2010. Malaria, about the disease. http://www.cdc.gov/malaria/about/disease.html , accessed 15.04.2010

Challand S, Willcox M, 2009. A clinical trial of the traditional medicine Vernonia amygdalina in the treatment of uncomplicated malaria. Journal of Alternative and Complementary Medicine 15, 1231-1237.

Deeni YY & Hussain HSN, 1994. Screening *Vernonia kotschyana* for Antimicrobial Activity and Alkaloids. Pharmaceutical Biology 32(4), 388-395.

Deng Y, 2007. *Fleroya,* a substitute name for *Hallea* J.-F. Leroy (*Rubiaceae*), 2007. Taxon 56, 247-248.

Diallo D, Marson A, Terreaux C, Touré Y, Paulsen BS, Hostettmann K, 2001. Screening of Malian Medicinal Plants for Antifungal, Larvicidal, Molluscicidal, Antioxidant and Radical Scavenging Activities. Phytotherapy Research 15, 401-406.

Diallo D, Maïga A, Diakité C, Willcox H., 2004. Malarial-5: Development of an antimalarial phytomedicine in Mali, in: H. Willcox, G. Bodeker, P. Rasoanaivo (Eds.), Traditional Medicinal Plants and Malaria, CRC Press, Boca Raton, pp. 117-130.

EEA (European Environmental Agency) 2010. http://glossary.eea.europa.eu/terminology/concept_html?term=primary%20metabolites accessed June 8th 2010.

Elliott S, Navakitbumrung P, Kuarak C, Zankum S, Anusarnsunthorn V, Blakesley D, 2003. Selecting framework tree species for restoring seasonally dry tropical forests in northern Thailand based on field performance. Forest Ecology and Management 184,177-191.

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

Fakae BB, Campbell AM, Scott IM, Teesdale-Spittle PH, Liebau E, Brophy PM, 2000. Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. Phytotherapie Research 14, 630-634.

Fish F & Waterman PG, 1972. Methanol-soluble quaternary alkaloids from African *Fagara* species. Phytochemistry 11, 3007-3014.

FAO (Food and agriculture organisation), 2008. Number of hungry people rises to 963 million. http://www.fao.org/news/story/en/item/8836/icode/ 9 Dec 2008, accessed 4th June 2010.

Garcia Prado E, Garcia Gimenez MD, De la Puerta Vásquez R, Espartero Sánches JL, Sáenz Roderiquez MT. 2007. Antiproliferative effects of mitraphylline, a pentacyclic oxindole alkaloid of Uncaria tomentosa on human glioma and neuroblastoma cell lines. Phytomedicine 14, 280-284.

Gessler MC, Nkunya MHH, Mwasumbi LB, Heinrich M, Tanner M, 1994. Screening Tanzanian medicinal plants for antimalarial activity. Acta Tropica 56, 65-77.

Gurib-Fakim A, 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine, 27, 1-93.

Halliwell B, 2005. Free Radicals and Other Reactive Species in Disease. Encyclopedia of Life Sciences©2005, John Wiley & Sons, Ltd. www.els.net, assessed May 25th 2010.

Hamza OJH, van den Beukel CJP, Matee HIN, Moshi HJ, Mikx FHH, Selemani HO, Mbwambo ZH, H. van der Ven AJAH, Verweij PE, 2006. Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections, J. Ethnopharmacol. 108, 124-132.

Hawkins B, 2008. Plants for life: Medicinal plant conservation and botanic gardens. Botanic Gardens Conservation International, Richmond, U.K.

Heinrich M, Barnes J, Gibbons S, Williamson EM, 2004. Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, London.

Heinrich M, Edwards S, Moerman DE, Leonti , 2009. Ethnopharmacological field studies: A critical assessment of their conceptual basis and methods. Journal of Ethnopharmacology 124: 1-17.

Hideyuki S, Toshinori K, Haruaki I, Frank M, Ayumi O, Jun'ichi K, 2003. Naucleamides A-E, new monoterpene alkaloids from *Nauclea latifolia*. Chemical & pharmaceutical bulletin 51, 58-61.

Hotellier F, Delaveau P, Pousset JL, 1980. Naucleidinal and epinaucleidinal, alkaloids of *Nauclea latifolia.* Phytochemistry 19(8), 1884-1885.

Kamatenesi-Mugisha M & Oryem-Origa H, 2005. Traditional herbal remedies used in the management of sexual impotence and erectile dysfunction in western Uganda. African Health Science 5, 40-49.

Kamikawa T, Hanaoka Y, Fujie S, Saito K, Yamagiwa Y, Fukuhara K, Kubo I, 1996. SRS-A Antagonist Pyranoquinolone Alkaloids from East African *Fagara* Plants and their Synthesis. Bioorganic and Medicinal Chemistry 4 (8), 1317-1320.

Katende AB, Birnie A, Tengnäs B, 1995. Useful trees and shrubs for Uganda. Regional Soil Conservation Unit (RSCU), Kenya.

Kato A, Moriyasu M, Ichimaru M, Nishiyma Y, Juma FD, Nganga JN, Mathenge SG, Ogeto JO, 1996. Isolation of alkaloidal constituents of *Zanthoxylum chalybeum* and *Zanthoxylum chalybeum* using ion-pair HPLC. Journal of Natural Products 59, 316-318.

Katuura E, Waako P, Ogwal-Okeng J, Bukenya-Ziraba R, 2007. Traditional treatment of malaria in Mbarara District, western Uganda. African Journal of Ecology 45, 48-51.

Keeley SC, Forsman ZH, Chan R, 2007. A phylogeny of the "evil tribe" (Vernonieae: Compositae) reveals Old/New world long distance dispersal: Support from separate and combined congruent datasets (*trn*L-F, *ndh*F, ITS). Molecular phylogenetics and evolution 44, 89-103.

Kokwaro JO, 1982. Flora of Tropical East Africa, Rutaceae. Part 2, Polhill RM (Ed.) A.A. Balkema, Rotterdam.

Kuglerova H, Marsik P, Vanek T, Kokoska L, Wanyana-Maganyi O, Van Damme P, Grade J, 2008. Antioxidative activity of Ugandan Medicinal Plants, Afr. J. Trad. Compl. Alt. Medicines, Abstract of the world congress on medicinal and aromatic plants, Cape Town November 2008, http://journals.sfu.ca/africanem/index.php/ajtcam/article/view/745 , assessed 07.04.2010.

Lartey A, 2008. Maternal and child nutrition in Sub-Saharan Africa: challenges and interventions. Proceedings of the Nutrition Society 67, 105-108.

Lindsay DG, Astley SB, 2002. European research on the functional effects of dietary antioxidants – EUROFEDA. Molecular Aspects of Medicine, 23, 1-38.

Lye KA, Bukenya-Ziraba R, Tabuti JRS, Waako PJ, 2008. Plant.Medicinal Dictionary for East Africa. Makerere Herbarium Handbook no. 2. Makerere University, Kampala, Uganda.

Martin GJ, 1995. Ethnobotany. Chapman & Hall, London.

Macko E, Weisbach JA, Douglas B, 1972. Some observations on the pharmacology of mitragynine. Archives internationals de pharmacodynamiie et de therapie 198, 145-161.

Matsumoto K, Horie S, Ishikawa H, Takayama H, Aimi N, Ponglux D, Watanabe K, 2004. Antinociceptive effect of 7-hydroxymitragynineinmice: Discovery of an orally active opioid analgesic from the Thai medicinal herb *Mitragyna speciosa.* Life Sciences 74, 2143-2155.

Matu EN & van Staden J, 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. Journal of Ethnopharmacology 87, 35-41.

Muganga R, Angenot L, Tits M, Frédérich M, 2010. Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in treatment of malaria, J. Ethnopharmacol 128, 52-57.

Nakatani M, Asai H, Mochihara K, Hase T, 1990. Jatrorrhizine chloride and other constituents from *Fagara chalybea* Engl. Rep. Fac. Sci. Kagoshime Univ., 23, 153-157.

Nergard CS, Diallo D, Michaelsen TE, Malterud KE, Kiyohara, Matsumoto T, Yamada H, Paulsen BS, 2004. Isolation, partial charachterisation and immunomodulating activities of polysaccharides from *Vernonia kotschyana* Sch. Bip. ex Walp. Journal of Ethnopharmacology 91, 141-152.

Ngnokam D, Ayafor JF, Connolly JD, Nuzillar JM, 2003. Nauclefolinine: a new alkaloid from the roots of Nauclea latifolia. Bulletin of the Chemical Society in Ethiopia 17, 173-176.

Nguta JH, Mbaria JH, Gakuya DW, Gathumbi PK, Kiama SG, 2010. Antimalarial herbal remedies of Msambweni, Kenya, J. Ethnopharmacol. 128, 424-432.

Noedl H, Wongsrichanalai C, Miller RS, Myint KS, Looareesuwan S, Sukthana Y, Wongchotigul V, Kollaritsch H, Wiedermann G, Wernsdorfer WH, 2002. *Plasmodium falciparum*: Effect of anti-malarial drugs on the production and secretion characteristics of histidine-rich protein II. Exp Parasitol 102, 157–163.

Okello J & Ssegawa P, 2007. Medicinal plants used by communities of Ngai Subcounty, Apac District, Northern Uganda. African Journal of Ecology 45 (Suppl. 1), 76-83.

Okoli AS, Iroegbu CU, 2004. Evaluation of extracts of *Anthocleista djalonensis, Nauclea latifolia and Uvaria afzalii* for activity against bacterial isolates from cases of non-gonococcal urethritis. Journal of Ethnopharmacology 92, 135-144.

Pearce RJ, Pota H, Evehe M-S B, Baˆ E-H, Mombo-Ngoma G, Malisa AL, Ord R, Inojosa W, Matondo A, Diallo DA, Mbacham W, van den Broek IV, Swarthout TD, Getachew A, Dejene S, Grobusch MP, Njie F, Dunyo S {, Kweku1M , Owusu-Agyei1 S, Chandramohan D, Bonnet M, Guthmann J-P, Clarke S, Barnes KI, Streat E 20, Katokele ST, Uusiku P, Agboghoroma CO, Elegba OY, Cisse´ B, A-Elbasit IE, Giha HA, Kachur SP, Lynch C,

John B, 2009. Multiple Origins and Regional Dispersal of Resistant dhps in African Plasmodium falciparum Malaria. PLoS Medicine, 6(4), 1-15. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2661256/pdf/pmed.1000055.pdf , accessed 16.04.2010

Pilarski R, Poczekaj-Kostrzewska M, Ciesiolka D, Szyfter K, Gulewicz K, 2007. Antiproliferative activity of various *Uncaria tomentosa* preparations on HL-60 promyelocytic leukemia cells. Pharmacological Repoerts 59, 565-572.

Prior RL, Wu X, Schaich K, 2005. Standarddized methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. J. Agric. Food Chem. 53, 42-4302.

Raschke V & Cheema B, 2008. Colonisation, the New World Order, and the eradication of traditional food habits in East Africa: historical perspective on nutrition transition. Public Health Nutrition 11, 662-674.

Razafimandimbison SG, Bremer B, 2002. Phylogeny and classification of *Nauclea*.s.l. (Rubiaceae) inferred from molecular (ITS, rBCL, and tRNT-F) and morphological data. American Journal of Botany 89(7), 1027-1041.

Rivier L, Bruhn JG, 1979. Editorial. Journal of Ethnopharmacology 1,1.

Rukunga GH, Gathirwa JW, Omar SA, Muregi FW, Muthaura CN, Kirira PG, Mungai GH, Kofi-Tsekpo WH, 2009. Anti-plasmodial activity of the extracts of some Kenyan medicinal plants. J. Ethnopharmacol. 121, 282-285.

Sachs J & Malaney P, 2002. The economic and social burden of malaria. Nature 415, 680-685.

Sandoval M, Okuhama NN, Zhang X-J, Condezo LA; Lao J, Angeles FM, Musah RA, Bobrowski P, Miller MJS, 2002. Anti-inflammatory and antioxidant activities of cat's claw (*Uncaria tomentosa and Uncaria guianensis*) are independent of their alkaloid content. Phytomedicine, 9, 325-337.

Sanogo R, Germano MP, de Tommasi N, Pizza C, Aquino R, 1998. Vernosides and androstane glycoside from *Vernonia kotchyana.* Phytochemistry 47(1), 73-78.

Seaton JC, Tondeur R, Marion L, 1958. The structure of mitraphylline. Canadian Journal of Chemistry 36, 1031-1038.

Shaik Mossadeq WM, Sulaiman MR, Tengku Mohammad TA, Chiong HS, Zakaria ZA, Jabit ML, Baharuldin MTH, Israf DA, 2009. Anti-inflammatory and antinociseptive Effect of *Mitragyna speciosa* Korth Methanolic Extract. Medical Principles and Practice 18, 378-384.

Shellard EJ, 1974. The alkaloids of Mitragyna with special reference to those of *Mitragyne speciosa,* Korth, United nations Office on Drugs and Crime, Bulletin on Narcotics, Issue 2, 41-55. http://www.unodc.org/unodc/en/data-and-analysis/bulletin/bulletin_1974-01-01_2_page005.html accessed April 22th 2010.

Shellard EJ & Lala PK, 1978. The alkaloids of *Mitragyna rubrostipulata* (Schum.) Havil. Planta Medica 33, 63-69.

Society of Ethnopharmacology, 2010. About us. http://www.ethnopharmacology.org/ISE_about_us.htm accessed May 25th 2010.

Ssegawa P, Kasenene JM, 2007a. Plants for malaria treatment in Southern Uganda: Traditional use, preference and ecological viability. Journal of Ethnopharmacology 27, 110-131.

Ssegawa P, Kasenene JM, 2007b. Medicinal plant diversity and uses in the Sango Bay area, Southern Uganda. Journal of Ethnopharmacology 113(3), 521-540.

Stangeland T, Tabuti JRS, Lye KA, 2007. The influence of light and temperature on the germination of two Ugandan medicinal trees. African Journal of Ecology 46, 565-571.

Stangeland T, Tabuti JRS, Lye KA, 2010. The framework tree species approach to conserve medicinal trees in Uganda. Submitted in March 2010.

Tabuti JRS, 2007. The uses, local perception and ecological status of 16 woody species of Gadumire Sub-county, Uganda. Biodiversity and Conservation 16, 1901-1916.

Tabuti JRS, Lye KA, Dhillion SS, 2003. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. Journal of Ethnopharmacology 88, 19-44.

Tabuti JRS, Ticktin T, Arinatwe MZ, Muwanica VB, 2009. Community attitudes and preferences towards woody species and their implications for conservation in Nawaikoke Sub-county, Uganda. Oryx 43(3), 393-402.

Taniguchi M, Haraguchi H, Higuchi F, Oi S, Chapya A, Kubo I, 1985. Biocidal Alkaloids from African *Fagara* Plants: Inhibition of Mitochondrial Function. Agricultural and Bilogical Chemistry 49(10), 3051-3052.

Traore F, Gasquet M, Laget M, Guiraud H, Di Giorgio C, Azas N, Doumbo O, Timon-David P, 2000. Toxicity and Genotoxicity of Antimalarial Alkaloid Rich Extracts Derived from *Mitragyna inermis* O. Kuntze and *Nauclea latifolia.* Phytotherapy Research 14, 608-611.

Traore-Keita F, Gasquet M, Di Giorgio C, Ollivier E, Delmas F, Keita A, Doumbo O, Balansard G, Timon-David P, 2000. Antimalarial Activity in Four Plants used in Traditional Medicine in Mali. Phytotherapy Research 14, 45-47.

University of Kent, 2010. Ethnobotany MSc Programme: Overview. http://www.kent.ac.uk/sac/prospective_students/courses/pgethno.html , accessed 15.04.2010

Vidal J. 2010. How food and water are driving a 21st-century African land grab. The Observer, March 7th 2010. http://www.guardian.co.uk/environment/2010/mar/07/food-water-africa-land-grab accessed June 6th 2010.

Villar J, Merialdi M, Gülmezoglu AM, Abalos E, Carroli G, Kulier R, de Oni M, 2003. Nutritional Interventions during Pregnancy for the Prevention or Treatment of Maternal

Morbidity, and Preterm Delivery: An Overview of Randomized Controlled Trials. J. Nutr. 133, 1606S-1625S.

Wagner H, Blatt S, 1996. Plant drug analysis. A Thin Layer Chromatography Atlas, second ed., Springer Berlin.

WFP (World Food Programme), 2010. Hunger. http://www.wfp.org/hunger , accessed 4th June, 2010

WHO/FAO 2003. Diet, nutrition and the prevention of chronic diseases. ftp://ftp.fao.org/docrep/fao/005/ac911e/ac911e01.pdf , pp. 23-25, accessed 7th June 2010

WHO, 2008. Traditional medicine. Fact sheet Nº134. http://www.who.int/mediacentre/factsheets/fs134/en/ accessed May 26th 2010.

WHO, (World Health Organisation) 2009. World Malaria Report 2009. http://www.who.int/malaria/world_malaria_report_2009/en/index.html , accessed on 5th May 2010.

Zirihi G N, Lengo, M, Frederic G-G, Bernard B, Phillippe G, 2005. In vitro antiplasmodial and cytotoxicity of 33 West African plants used for treatment of malaria. Journal of Ethnopharmacology 98, 281-285.

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Recognition and development of traditional medicine in Tanzania

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Abstract

The aim of this paper is to trace developments in Traditional Medicine (TM) and legislation concerning conservation and use of biodiversity in Africa, with Tanzania as a case study. Based on field trips, interviews with different actors, site visits, and literature we explored the history, current status, re-establishment, and development of TM. A summary of laws and regulations concerning forests, access and benefit sharing is presented. During the last decade the Government of Tanzania put forth legislation to address national health needs, traditional knowledge, and the resource base for TM (e.g., practitioners, biodiversity). Our findings indicate that TM is the most common form of health care, and that the HIV pandemic has highlighted the need to work across health sectors. New legislation has facilitated this need. In Tanzania TM is experiencing a renaissance in being formally recognized, integrated into mainstream health care, formal establishment of practitioners, and gaining the interests of different sectors. More studies on bioactivity, safety, domestication, and sustainability of use of medicinal plants are needed. Development of TM can also, other than making a significant contribution to health care and livelihoods, provide income possibilities. It is however yet to be seen if the recent regulations can be made fully operational and implemented.

Keywords: Bioprospecting; Biodiversity conservation; Community-based management of natural resources; Ethnobotany; Indigenous knowledge; Traditional medicine

1. Introduction

Traditional medicine (TM) is the diagnosis and treatment of psychological and medical illnesses based on local knowledge and socio-cultural and religious beliefs, developed over time by local people within their belief systems and specific environmental (particular biodiversity) conditions of a particular area (Grenier, 1998; Diallo and Paulsen, 2000; Tabuti et al., 2003). It is a well-established system of medicine, parallel to the western or orthodox medicinal system, still in active use by rural communities in developing countries (Iwu and Laird, 1998; Tabuti et al., 2003). Due to the lack of proper conventional health care systems, TM is often the first choice for providing primary health care. In Tanzania (Fig. 1), the accessibility to conventional medical doctors is very low (1:33,000) compared to that of traditional medicine practitioners (TMPs) (1:350–450) (Marshall, 1998; IRIN, 2006).

In Africa, during occupied periods colonial powers connected TMPs to the use of supernatural forces or witchcraft, and TM was subject to discredit and legal bans. When colonization ended, independence made some nations more tolerant towards TM, regaining African identity and developing national and cultural values. Two nations fully incorporating TM in their health care systems are Ghana and Mali (Diallo and Paulsen, 2000; Romero-Daza, 2002). Other nations like the Ivory Coast, Comoros, Seychelles and Cape Verde are less favourable towards TM:

Abbreviations: ABS, access and benefit sharing; CBD, Convention of Biological Diversity; CITES, Convention on International Trade on Endangered Species; CRFs, catchment reserve forests; DMT, Département de la Médicine Traditionelle; GDP, gross domestic product; GURT, Government of the United Republic of Tanzania; ITM, Institute of Traditional Medicine; MAPs, medicinal and aromatic plants; MNRT, Ministry of Natural Resources and Tourism; MPs, medicinal plants; NFP, National Forest Programme; NGOs, non-government organizations; PAs, protected areas; PIC, prior informed consent; TAWG, Tanga AIDS Working Group; TM, traditional medicine; TMPs, traditional medicine practioners; TPI, Tanzanian Pharmaceutical Industries; WHO, World Health Organization.

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Fig. 1. Map of Tanzania. (See ref. University of Texas and Libraries, 2007).

TMPs are not involved in the official health system and no regulations exist for their registration or licensing. Other countries, like Angola and the Central African Republic, have established systems for registration of TMPs but do not officially recognize their practices.

Most of the 45,000 TMPs in Ghana are recognized and licensed in various associations under the umbrella of the Ghana Federation of Traditional Medicine Practitioners' Association. The Traditional Medicine Unit was established as part of the Ministry of Health in 1991, working directly with TMPs (Romero-Daza, 2002). In Mali the Phytotherapy Institute was established in 1968 as the first research establishment for the study of medicinal plants (Diallo and Paulsen, 2000). After several changes, the establishment is now called the Department of Traditional Medicine (Département de la Médecine Traditionelle, DMT). DMT became a collaborating centre of the World Health Organization (WHO) for research in TM in the early 1990s. One of the primary objectives of DMT is to establish a mechanism to assure that TM becomes complementary to conventional medicine. In South Africa the governmental health service only provides western medicine (Light et al., 2005), but TM is still used by the majority of people, especially in rural areas. The new government and National Research Foundation, however, are now promoting more research on natural resources, and have allocated more funding to studies in Indigenous Knowledge Systems. This promotion had precipitated in significant increase in research, e.g., in the last 10 years the number of publications from South Africa in the Journal of Ethnopharmacology increased from about 20 to 55% of all African publications.

Internationally, TM has received much attention the last decades. In 1977 the World Health Assembly urged member states to utilize their traditional systems of medicine (resolution WHA30.49). In 1978 the International Conference on Primary Health Care, held in Alma-Ata, recommended that governments give high priority to the utilization of TMPs and Traditional Birth Attendants, and incorporate proven traditional remedies into national drug policies and regulations (Akerele, 1987) (Table 1). During the nineties several conferences and meetings on the topic were held in Tanzania and other African countries, starting with the International Conference of Experts from Developing Countries on Traditional Medicinal Plants, Arusha, February 1991. In a Meeting of the Inter-African Experts Committee on African Traditional Medicine and Medicinal Plants in the Organization of African Unity (OAU) the Decade of African Medicine was proclaimed from 2001 to 2010 (Mahunnah, 2002), and the African Traditional Medicine Day was set to be on the 1st of September. Tanzania celebrated the day for the first time in 2003. The urgency of recognizing TM has been further heightened by the HIV/AIDS pandemic (Romero-Daza, 2002) as many HIV positive individuals use herbal remedies to boost the immune system and to fight opportunistic diseases (Scheinman, 1998; IRIN, 2006).

Recent studies indicate the importance of TM among rural people in Africa today (Jäger and van Staden, 2000; Light et al., 2005) and that legislation concerning conservation/use of biodiversity and TM are increasingly adopted (Diallo and Paulsen, 2000; Romero-Daza, 2002). Tanzania we believe is one of the countries that has been championing TM and its practice, and in fact may be an example of good practice. We use it as a case study tracing developments in recognition, facilitation, and re-establishment of TM from current and historical perspectives. In light of the Convention on Biodiversity (CBD) and appreciating the close link between wild plant species and traditional medicine, the current status of biodiversity, conservation/forest legislation, and access and benefit sharing (ABS) to biodiversity in Tanzania is described briefly, followed by a comprehensive overview of the current status of traditional medicine in Tanzania. The case sheds light on salient processes and links that have been core to the 're-'establishment of TM in Tanzania.

1.1. Methods

Informal and semi-structured interviews and group meetings with NGOs, traditional healers, medicinal plants collectors and researchers were conducted along with an in-depth literature survey. Field information was gathered during three visits to Tanzania in February 2002, October 2003 and August 2004. All information has been kept updated until the submission of this paper through communication with local informants. A chronology of related events in relation to TM and biodiversity was constructed and is provided in Table 1.

2. History, biodiversity, legislation and trade

The Republic of Tanganyika was formed in 1962, with Julius Nyerere as president (Table 1). Tanganyika and Zanzibar merged in 1964 to form Tanzania. Nyerere's political programme was socialistic, founded on African collectivistic traditions and village community. Up to the mid-1970s, the economy of Tan-

Table 1
Historical events in Tanzania concerning biodiversity conservation and traditional medicine

Year	International	Tanzania		
		General history	Biodiversity conservation	Traditional medicine
2005				The Traditional and Alternative Medicine Act is operational
2004			The 2004 Forest Note	
2003				TPI launched an anti-malarial medicine
2002			The Forest Act 2002	The Traditional and Alternative Medicine Act
2001	OAU proclaimed the decade of African TM			
1998				TPI privatised
1992	CBD, 05.06.			
1991	Intern. Conf. of Experts from Dev. Countries on TMPs			The Institute of Traditional medicine (ITM)
1990				The Tanga Aids Working Group (TAWG)
1989			Community-based conservation	
1987				The National Tree Seed Programme
1986		Economic recovery programs		
1978	WHO: Alma-Ata conference			Tanzanian Pharmac. Industries (TPI)
1974		Economical problems		Traditional Medicinal Research Unit (TMRU)
1964		Tanganyika and Zanzibar merges to Tanzania		
1962		Republic, J. Nyerere president		
1961		Independence, member of UN		
1929				Medical Practioners and Dentist Ordinance
1928				The Witchcraft Ordinance
1921			Kilimanjaro Forest Reserve	
1919		Great Britain took over Tanganyika		
1904			Forest Conservation Ordinance: 3/4 million ha converted to Forest reserves	
1882		The Germans colonized Tanganyika		
	Precolonial time			TM only medical system

Between 1991 and 1998 several NGOs mentioned in the text were established (e.g., The Dakika group, Envirocare and Honamed).

zanian mainland was performing modestly well. Then problems began to arise. Agricultural and industrial production declined, partly due to the falling export prices for primary products and the rising import prices. Internal causes were an economic policy not stimulating growth, and for agriculture: recurring drought. Since 1986 the country has been through a number of Economic Recovery programmes. The economy relies heavily on agriculture, contributing to almost half of Tanzania's gross domestic product (GDP); providing 85% of export earnings and employing 80% of the labour force. The economic growth is now 6.1% (2005), with a GDP per capita of US$ 700, among the lowest in the world (CIA, 2006). The population size of mainland Tanzania tripled from 13.6 millions in 1970 to 36.8 millions in 2005, and 80% of the population now lives in rural areas. With a population growth on 1.8% per year, Tanzania is currently experiencing an increasing land scarcity, shorter agricultural rotations, declining yields, and consequently a substantial increase in the pressure on remaining stocks of forestland (Kilahama, 2003; MNRT, 2003).

The health situation is severely worsened as the HIV/AIDS epidemic has spread. The first cases were reported in 1983, and it is now estimated that the percentage of population infected by HIV is 8.8% (estimated in 2003, CIA, 2006). The exact numbers are uncertain, and it is estimated that only one of five cases are reported. The epidemic has had a serious impact on the county's economy, mainly affecting the most economically active group of adults, those aged 15–45, and also a large proportion of the poor.

2.1. Status of biodiversity

Tanzania covers an area of 945,000 km^2, of which the forested area constitutes 40–60% depending on the definition of "forest", see Table 2; most of it is Miombo woodlands. The Central Government Forest Reserves comprise 10 million ha, of which 1.6 million ha are Catchment Reserve Forests (CRFs), and 3 million ha Local Government Reserves. The Eastern Arc forest represents one of the oldest and most stable terrestrial ecosystems on the continent, and is recognized to be one of the 25 biodiversity hotspots of the world, with a substantial number of endemic species. In a governmental report on biodiversity, the country is divided in six ecological zones (Table 2). All zones are exposed to degradation (GURT, 1998). The most important reasons for deforestation are conversion to agriculture and fuel wood consumption. It is estimated that fuel wood accounts for 97% of all fuel consumption and 92% of the country's source of energy. The average degradation level in CRFs is 18.5%. In South Kilimanjaro CRF substantial illegal activities are linked to pit sawing of hardwood such as camphor and cedar for carpentry workshops in towns and for illegal trade. The World Bank (2001, in MNRT, 2003) sums up challenges facing Tanzania's forest policy, related to weak oversight for forest and woodland management.

The Coastal forest is botanically rich, but 90% of original forest is destroyed (Table 2). Twenty percent of the forests are reserves, but many of them are too small to secure species survival. The mountain forest constitutes only 6.1% of total land area, but is very rich in flora. Of 4000 plant species, 75% are endemic. Nearly 30% of the mountain forests are reserves, 70% of the land outside reserves has been converted to farmland, grazing pastures or is degraded. The Brachystegia-Jubernadia Woodland (Miombo) covers almost 60% of the land area, nearly half of it being within protected areas (PAs). It is rich in flora with 8500 species of plants, 54% of which are endemic. Over 20% of the woodland is converted to farmland, grazing or degraded.

2.2. Legislation: forest, access and benefit sharing

In the past few years several new policies, laws and regulations have been adopted in Tanzania. The overall goal of the 1998 National Forest Policy is to "enhance contribution of the forest sector to sustainable development and the conservation and management of natural resources for present and future generations" (MNRT, 1998, see details in Kabudi et al., 2002).

The main objectives of the Forest Policy are: (i) ensured sustainable supply of forest products and services by maintaining sufficient forest area under effective management; (ii) increased employment and foreign exchange earnings through sustainable forest-based industrial development and trade; (iii) ensured ecosystem stability through conservation of forest biodiversity, water catchments and soil fertility; (iv) enhanced national capacity to manage and develop the forest sector in collaboration with other stakeholders (MNRT, 2003).

To implement the policy, two instruments have been used: (i) the National Forest Programme (NFP) commissioned in 2000 and (ii) forest legislation—the Forest Act of 2002. In April 2002 the Parliament approved the Forest Act 2002 (MNRT, 2002). It was made operational from 1 July 2004, when the Forest Regulations of 2004 were also approved. The Regulations provide arrangements on prior informed consent (PIC) and provisions for ABS related to genetic resources (MNRT, 2004). All access to resources in forests governed by the Forest Act shall be subject to an application for a prior informed consent (PIC) to the Director and the Forest manager. The application form is prescribed in the Regulations (29 Schedule). The application is to be published

Table 2
Characteristics of the Tanzanian ecological zones; botany and medicinal plants (MPs)

Ecological zone	% of total land area	% area within PAs	Biodiversity quality and MP richness	Relative change
Z I: moist forest mosaic	4.4	12.0	Rich in plant sp.; poor in endemic plants	Heavy human pressure due to cultivation, grazing and fuelwood; more than 20% of forest area has been lost
Z II: coastal forest	6.2	21.2	Rich in plant species and MPs rich, 600 endemic sp., habitat fragmentation threatens species survival	Over 90% of original forest destroyed; many FRs is too small to be viable as PAs
Z III: mountain forest	6.0	27.7	Rich in flora and MPs, of the 4,000 plant species, 75% endemic; 1/5 of tree sp also endemic	More than 70% of land outside PAs is converted to farmland, grazing or is degraded
Z IV: acacia-Savannah Grassland	18.1	41.4	Moderately rich in flora and MPs, 2500 species of plants	Extensive areas outside PAs suffer severe deterioration due to overstocking
Z V: acacia-Commiphora Thornbush	7.2	37.2	Moderately rich in flora, 2500 sp. of plants of which 50% are endemic	Extensive areas outside PAs suffer severe deterioration due to overstocking
Z VI: brachystegia-Jubernadia Woodland (Miombo)	58.1	46.7	Very rich in flora and MPs, 8500 sp. of plants of which 54% are endemic; famous for fine hardwoods	Over 20% of woodland has been converted to farmland, grazing or degraded; extensive deforestation for charcoal, fuel wood and overgrazing occurs

Adapted from: Stuart et al. (1990) and Clark (1995) in GURT (1998) GURT (Government of the United Republic of Tanzania) 1998.

in the *Gazette* or in a newspaper that is reasonably accessible to the public for 90 days. Any person may comment on the application. The Director can grant access only if all requirements under the Regulations have been fulfilled. An access permit is only valid if there is a written PIC. There are regulations on commitments to be undertaken by the collector, for example, (i) to adhere to limits on quantity and quality of biological resources for export; (ii) deposition of duplicates with all information to governmental agencies; (iii) not to apply for any form of intellectual property protection; (iv) sharing of knowledge and benefits. The access permit is subject to the payment of a fee made before a collection can be done. The state shall ensure that at least 50% of the benefits should be channelled to the concerned local community. The implementation and operationalization of this apparently comprehensive Forest Act is yet to be seen.

2.3. Trade in MAPs

Vendors are present in most urban centres in Tanzania. In Dar es Salaam, about 90 vendors were recorded in two markets, most of them being Maasai women. They were selling powdered plant material. Maasai men who transported plant medicines in 50 kg sacks supplied these vendors, who then sorted and prepared medicines for sale (Marshall, 1998). New groups like Honamed and Dakika (see Section 3.7) are selling their products at national and regional fairs and exhibitions as well as in their own clinics/shops.

Tanzania formerly exported medicinal plants, but no information exists on quantities or species traded, as there is no written legislation pertaining to these issues. One taxon thought to be over-exploited for international trade for the cosmetic industry is *Osyris* sp., harvested in the Kilimanjaro region. A Dar es Salaam-based exporter of *Prunus africana* (Hook.f.) Kalkman has been located, reported to harvest up to 120 t per year. No trade of the species is reported to CITES, despite the taxon's Appendix II-listing since 1995. Annual harvesting of the bark was estimated to be 3.500 MT in 1997 (Pierce and Laird, 2003). Some species are known to be exported from the Tanga region for research into their potential against AIDS. Nine medical plant species are reported to be of conservation concern in Tanzania (Marshall, 1998). It is difficult to get reliable and updated information on international trade in medicinal plants from Tanzania as there is no legal or formally organized export from the country.

2.4. National Tree Seed Programme

The National Tree Seed Programme is a governmental body established in 1987. The activities of the National Tree Seed Programme are directed towards establishment of seed sources, procurement, and supply of high quality tree seed. Its aim is to meet immediate as well as future seed demand from all growers. The Programme has an Arboretum, three medicinal plant gardens and long experience on propagation and domestication of indigenous trees, and can now offer seeds from 130 indigenous tree species (www.tanzania.go.tz, 2006).

3. Current status of traditional medicine

3.1. Traditional medicine and regulations

During colonization and up to the present TM has not been officially accepted. Under the British rule, provision for TM practice was given through Medical Practitioners and Dentist Ordinance, 1929, but cooperation between TMPs and conventional doctors was forbidden. The Witchcraft Ordinance of 1928 was operational until 2002, forbidding witchcraft. However during the period 1970–1988, approximately 3690 people reportedly died in witchcraft-related incidents (Marshall, 1998).

Tanzania is now in the process of recognizing the TMPs and incorporating their practice in the health sector. In 2002 The Parliament passed The Traditional and Alternative Medicine Act, replacing the old laws (Table 1). The new legislation aims at integrating traditional medicine in the national health care system, and encourages cooperation between traditional healers and physicians. It also provides protection against piracy of the traditional healers' products (Kauye, 2002). Traditional medicine is now under the Ministry of Health. In 2005 the new Act finally became operational.

3.2. Traditional knowledge and bioprospectors

Knowledge and identities of medicines are usually kept as personal secrets or within certain expert circles of TMPs. Bioprospectors have extracted knowledge by different means and contracts with or without benefits to the owners of the knowledge (see discussions and cases in ten Kate and Laird, 1999; Svarstad and Dhillion, 2000; Kabudi et al., 2002). In the case of Tanzania the earliest cases include the export of Busy Lizzie (*Impatiens sultani* Hook.f.) in the middle of the 19th century and the African Violet (*Saintpaulia* spp.) reportedly exported in 1893. These plants bring high amounts of profit to the actors in the North without any benefits of the shares appropriated to Tanzania. Today, however, contracts can be drawn to ascertain that benefits from the use of biological diversity and traditional medicinal knowledge are channelled to the South, ensuring equitable benefit sharing as recommended in the CBD (Juma, 1989; Svarstad and Dhillion, 2000; Laird, 2002; Nelson-Harrison et al., 2002; CBD, 2004; MNRT, 2004). In Tanzania detailed benefit sharing procedures have been worked out in The Forest Regulations, 2004 (see Section 2.2).

3.3. The Institute of Traditional Medicine (ITM)

ITM was founded in 1991 as a successor of the Traditional Medicine Research Unit founded as a part of the University in Dar es Salaam in 1974. The Traditional Medicine Research Unit had two broad objectives: to seek materials of plant and animal origin that might be of medicinal value, and to establish a record of cultural significance in Tanzania Society of the Traditional Healers and its role in the village. ITM and its precursor has performed and taken part in numerous ethnobotanical studies (Chhabra et al., 1984, 1987, 1990a,b, 1993; Hedberg et al., 1982, 1983; Johns et al., 1994; Schlage et al., 2000). Today ITM conceives a future in which it will have a prominent role in research

and development of medicinal plants by helping the country to produce raw materials for plant-derived medicines, standardizing herbal medicines and biological testing. WHO AFRO has identified the priority areas for research in this decade to be malaria, HIV/AIDS, hypertension, sickle cell anaemia and diabetes mellitus. The main focus of ITM at present is on the testing for antiviral, anticancer and antiprotozoan activities and some of the opportunistic conditions of HIV/AIDS. The Institute is also planning to build capacity for the cultivation and extraction of *Artemisia annua* L. for production of Artemisin. Production of community-based cultivation is looked upon as a good strategy for poverty alleviation and at the same time supplying plant material for local use and export.

3.4. Ethnobotanical knowledge, research and industry

Considerable ethnobotanical research has been done in Tanzania. About 45% of the country is said to have been studied ethnobotanically (Mahunnah, 1996 in GURT, 1998). Most of the endemic medicinal plants are found in the moist forests, particularly in the mountains and on the eastern coast. The lowland woodlands, thickets and grasslands are also rich in medicinal plants (GURT, 1998). Most of the studies describe plants and their utilization (e.g. Hedberg et al., 1982, 1983; Kabudi, 1990; Chhabra et al., 1993; Merker, 1904). In the 1980s The Traditional Research Unit (now ITM) and the Department of Chemistry at the University in Dar es Salaam conducted comprehensive work on screening plants for active compounds (Chhabra et al., 1984; Weenen et al., 1990). The work continued through the decade with the registration of plants used in Eastern Tanzania supplemented with results of a literature survey on medicinal uses, isolated constituents and pharmacological effects (Chhabra et al., 1987, 1990a,b, 1993). Recently there have been several studies on bioactivity and screening for active compounds (e.g., Fyhrquist et al., 2002, 2004; Beha et al., 2004; Moshi et al., 2004; Mbwambo et al., 2004; de Boer et al., 2005).

Another trend in the studies is gradually increasing attention on frequency of use and sustainability of harvesting. In one of the studies (Johns et al., 1994) among the Batemi people in north-central Tanzania, a quantitative interaction effect was calculated for each remedy. They used a log linear model developed by Johns et al. (1990) where the interaction of i and j, which indicates the potential of a plant i as a cure for disease j, is a quantitative measure of the degree of confirmation of any particular remedy. In a more recent study by Schlage et al. (2000) documentation and ethnopharmacological evaluation of medicinal plants of the Washambaa people in the Western Usambara Mountains were performed. A total of 328 taxa were collected, yielding 2260 individual use reports. The use reports were arranged into nine groups of medicinal uses based on the illnesses treated. A Factor of Informant Consensus was used in order to evaluate the ethnobotanical importance of the plants. For the most commonly used taxa an ethnopharmacological evaluation was performed. Studies to evaluate the effectiveness as well as toxicological data are still lacking for many of the species.

In the study of the Batemi people (Johns et al., 1994) the authors stress that they were particularly interested in the sustainable use of the medicinal plants. They refer to another ecological study performed in the area (Smith, 1993), showing that half of the medicinal plants in the study were locally rare or located far from the Batemi communities. Four of the species were found to be in need of conservation attention in the area.

A larger emphasis on sustainable utilization and conservation of tropical woodlands is expressed in a study of different utilization of trees and distribution of ethnobotanical knowledge in Morogoro, Eastern Tanzania (Luoga et al., 2000). The major type of vegetation was Miombo woodland. The overall aim of the study was to ascertain the local people's knowledge of and reliance on woody resources as a first step towards sustainable resource conservation. A total of 133 arborescent species in 31 families was identified and classified into 12 categories of use. Major uses were charcoal, firewood, medicine and poles. A use value analysis was performed on all species; uses were categorized in three classes: no use (0), minor use (0.5) and major use (1). The results showed that 69% of the species had uses. Only 13 of 37 families recorded had use values greater than 1, whereas 6 families had use value 0. This indicated that the intensive and multiple uses were focused on few species/families. The study concludes that the harvest of the exceptionally useful species may far exceed their regeneration and production.

There have been several industries that have emerged as TM has been more discussed in the main stream. Here we give details on two companies to illustrate that sophisticated extraction and processing is carried out not in Tanzania. Tanzanian Pharmaceutical Industries (TPI) was originally created as a state business in 1978. The new private owners took over the plant in 1998 and have since renovated and modernized the installations. In September 2003, TPI launched an anti-malaria medicine based on the active ingredient in the plant *Artemisia annua* L. and expects to obtain a big share of the anti-malaria medicine market in Africa with this product. The product is a result of a joint venture between TPI, a Thai group, and the German NGO Action Medior. Recently TPI has expressed great interest in entering into something similar with any interested party. TPI however cannot buy medicinal plants directly, as it does not have an extraction plant. African Artemisia Ltd. is another company that grows the plant *Artemisia annua* in Arusha on a grand scale (plantation style). The quality of the *Artemisia annua* grown in Arusha is supposedly very good due to the volcanic soil. The plants are dried at the plant and sent to Belgium where the ingredients are extracted and to be sold to the pharmaceutical industry.

3.5. Management of medicinal plants

If the effectiveness of MPs gets widely known and the availability is improved, over harvesting and extinction of plants can result (Jäger and van Staden, 2000; Dhillion and Amundsen, 2000; Sparg et al., 2005). Some studies indicate that indigenous management systems (and community-based management) can be valuable for enhancing biodiversity conservation (Kivumbi and Newmark, 1991; La Frankie, 1994;

Michon and De Foresta, 1997; Mgumia and Oba, 2003; Shrestha and Dhillion, 2003; Dhillion and Gustad, 2004). Such systems should be encouraged. In planning further ethnobotanical studies, both descriptions of plants, harvesting, population dynamics, and traditional management systems should be performed. When harvesting methods prove not to be sustainable, possibilities for enrichment planting or domestication should be explored (Mukherjee and Wahile, 2006).

3.6. Information on TM research

There have been several donor-funded projects to gather and publish information on TM. Following are two examples that have made significant contributions. In a project running over 3 years in the 1990s, funded by the Canadian International Development Agency (CIDA), the United States Department of Agriculture (USDA), and the Food and Agriculture Organization of the United Nations (FAO), activities were undertaken to assess rural people's needs for tree products and to match needs and species preferences with appropriate silviculture and forest management practices. The project resulted in the following: (i) a framework for assessing species of value to local people that have a potential for more intensive cultivation; (ii) a handbook: "Indigenous Multipurpose Trees in Tanzania" (Eckman and Hines, 1993, available on FAO, 2006); (iii) SPECIES, a user-friendly computer programme, developed as a data base for tree species information. Recently an extensive handbook: "Edible Wild Plants of Tanzania", funded by the Regional Land Management Unit (RELMA) and the Swedish International Development Cooperation Agency (SIDA), has been published (Ruffo, 2002). Approximately 60% of the plants in the book are also used as medicine. In the book there is a report from a workshop held in Iringa in November 2002 that drew various actors and disciplines. They suggested several activities for the promotion of use, propagation and domestication of edible and medicinal plants in Tanzania.

3.7. New groups working with medicinal plants

In the 1990s there has been a surge of individuals and companies working with medicinal plants at the local and national scale. We give examples of the few that we studied.

The Dakika Medicine Plant Growing Group in Kikatiti, Arusha, was organized in 1991 by a local NGO with some external help. The group focuses on growing the Neem tree (*Azadirachta indica*) for making organic pesticides and to use as ingredients in traditional medicines. They appear to be well organized and have a sound economy coming from selling plants (various trees beside Neem), seeds for medicinal plants and medicines, herbs and nutritional mixtures for children. The group has contact with local and national institutions like the Tanzanian Wild Life Research Institute and the Ministry of Forestry who provide them with Neem-seeds from different regions of Tanzania and plastic-bags for growing the seedlings. The group is member of the Tanzanian Herbalist Organization and attends national and international conferences and exhibitions on traditional medicines. With the introduction of the new law of Traditional medicine in 2002, the healers and groups like Dakika now are under the Department of Health. This was seen as fortunate for the sector as it would increase study and research on the medicinal effects of local trees and plants. According to the group the market for traditional medicine is quite good with reasonable prices. Most of the products are sold directly to users at exhibitions and congresses or from their clinic in Kikatiti, while some are sold to middlemen.

HONAMED (the House of Natural Medicine) was founded by Dr. Meyenjwa Rugina in 1998. HONAMED has a small workshop where plants are processed and various medicines produced with the help of some simple machinery and three employees. The company is represented in conferences and exhibitions in Tanzania and the neighbouring countries. Marketing is easy even though the products are quite expensive. We were shown plastic bags of powdered *Artemisia annua* used against malaria that are sold for Tsh. 5000 a packet (US$ 5 per treatment). In addition many of the medicines are based on extracts from the Neem tree that is growing abundantly in the area since the government in the beginning of the nineties promoted the planting of one tree per family in the area. New mixes of medicines are also experimented with. There is interest in investing in machinery for extracting oil, better dryers, and milling machines, however it is difficult to get financing for this type of activity and the existing micro-finance is very expensive (with interest rates as high as 35–40%).

3.8. Cooperation between TMPs and primary health sector

Generally cooperation between TMPs and the health sector did not exist in Tanzania as in most other African countries. According to the old laws cooperation between physicians and TMPs was forbidden. For example, the Tanga AIDS Working Group (TAWG) started 'by accident' in 1990. The German doctor, Elmar Ulrich, provided by the German Development Service, was working at a hospital in Pangani District. He noticed that many of his patients visited TMPs prior to going to hospital, and that some were overdosed. He realised that the healers were treating people with strong substances and decided to contact them with the aim of starting a referral network. The healers responded positively and the referral group became the start of the present day TAWG. The objective of TAWG is to reduce transmission of HIV and assist people with AIDS. TAWG is an interdisciplinary group, which includes conventional physicians, service providers, traditional healers, patients, social scientists and botanists. TAWG collects plant medicines from traditional healers and distributes the medicines to their patients. They have offices in Bombo Regional Hospital, and in two other District Hospitals. TAWG also carries out ethnobotanical research; they distribute three plant-based medicines to other areas, and in the coming years aim at identifying at least 10 new efficacious medicines (Scheinman, 1998; IRIN, 2006). TAWG is a good example of fruitful cooperation between allopathic medicine and THs.

4. Conclusions

This review and study shows that TM has been progressively paid attention to and is included in the development plans of Tanzania. The Government has decided to encourage the integration of TM into the primary health system. In 2002 The Parliament passed *The Traditional and Alternative Medicine Act*, which became operational in 2005 with the aims of integrating TM in primary health care and encouraging cooperation between TMPs and western trained doctors. The Traditional Healers are the very source of knowledge on medicinal plants. Respect for the knowledge of Traditional Healers and operational regulations for benefit sharing as shown by the well-known case of Mali are regarded as an essential basis for fruitful cooperation across sectors. Here it appears that there are still strides to take in Tanzania but the prognosis is optimistic. Although plant species may occur across political borders their specific use and preparation, and cosmological place in each local community is unique and should be protected as alluded to by the CBD: uniqueness of use is not cosmopolitan. Species occurring across borders and having overlapping use does not justify non-recognition of local knowledge that has led to their know-how: regional and cross-border negotiations must take into account local uniqueness.

Two of the botanically most important ecological zones in Tanzania; Coastal forests and Mountain forests, suffer from severe degradation, 70–90% of forests outside Protected Areas being destroyed. Much of the medicinal plants are found in these zones. In new regulations and laws, the aim is to use the genetic resources for the benefit of present and future generations. The main legal framework seems to be in place, but the slow procedures of making the laws operational endanger its accomplishment. In addition, legislation related to the international trade in plants should be clarified and cooperation between agencies to implement existing laws should be promoted.

The world market in MAPs is estimated around US$ 60 billions, and in countries like China and India this trade is becoming increasingly important. In Africa there is a considerable informal trade in MAPs from TMs and in markets, but it is difficult to estimate the value of this 'hidden economy'. Experience from China, India and South Africa shows that sustainable harvesting is not sufficient to save threatened species, and in China and South Africa there are initiatives to cultivate MAPs. Tanzania is quite well mapped in descriptive ethnobotanical studies. However there are few papers on population measurements, harvesting regimes, sustainability of harvesting and pharmacological studies. Before TM is to be developed further and incorporated in primary health care, it is important to intensify studies on populations and sustainability of harvesting of medicinal plants. Sustainable harvesting and growing of medicinal plants have the potential of accelerating rural development. Some local stakeholders have already started a process that can contribute to this development. Pharmacological studies to confirm safety and effectiveness of medicinal plants are now being done, but still very few plants have been screened.

Recent visits in Tanzania showed increasing efforts on the development of TM and modern phytomedicines. The Dakika group, for example, had for several years successfully been growing, harvesting and processing both exotic and indigenous MPs, and bringing the products out into a bigger marked. Their problems were poor equipments and labour-intensive methods. The Tanzanian Pharmaceutical Industries was newly renovated and had just launched a new anti-malaria medicine based on locally grown *Artemisia annua* L. However there are no extraction plants in Tanzania, so the plant material has to be dried and sent to Belgium to extract the active compounds. An increase in capacity and funds in developing more sophisticated approaches within the country would be of importance for both small and large industries.

It currently appears that several laws and regulations in Tanzania are being put in place as a result of commitments to the Convention on Biological Diversity (CBD), the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and poverty eradication plans. Furthermore there are serious endeavours to include TM in primary health care also triggered by legislation. It is however a long process for the laws and plans to become operational and implementation to take place. It is also a question if the large number of laws and regulations will make implementation tedious and bureaucratic, and hinder the development of the R&D sector. The implementation of laws is difficult without capacity building, which requires serious consideration.

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References

Akerele, O., 1987. The best of two worlds: bringing traditional medicine up to date. Social Science and Medicine 24, 177–181.

Bally, P.R.O., 1938. Heil und giftpflanzen der Eingeborenen von Tanganyika. FEDDEs Repert.speci. nov. regni veget. Beihefte 102, Berlin-Dahlem, 1–81.

Beha, E., Jung, A., Wiesner, J., Rimpler, H., Lanzer, M., Heinrich, M., 2004. Antimalarial activity of extracts of *Abutilon grandiflorum* G.Don—a traditional Tanzanian medicinal plant. Phytotherapy Research 18, 236–240.

CBD, 2004. Convention on biological diversity website. http://www.biodiv.org/default.aspx (accessed on November 1, 2004).

Chhabra, S.C., Uiso, F.C., Msiu, E.N., 1984. Phytochemical screening of Tanzanian medicinal plants. Journal of Ethnopharmacology 11, 157–179.

Chhabra, S.C., Mahunnah, R.L.A., Mshiu, E.N., 1987. Plants used in traditional medicine in Eastern Tanzania. I. Pteridophytes and Angiosperms (Acanthaceae to Canellaceae). Journal of Ethnopharmacology 21, 253–277.

Chhabra, S.C., Mahunnah, R.L.A., Mshiu, E.N., 1990a. Plants used in traditional medicine in Eastern Tanzania. III. Angiosperms (Euphorbiaceae to Menispermaceae). Journal of Ethnopharmacology 28, 255–283.

Chhabra, S.C., Mahunnah, R.L.A., Mshiu, E.N., 1990b. Plants used in traditional medicine in Eastern Tanzania. IV. Angiosperms (Mimosaceae to Papilionaceae). Journal of Ethnopharmacology 29, 295–323.

Chhabra, S.C., Mahunnah, R.L.A., Mshiu, E.N., 1993. Plants used in traditional medicine in Eastern Tanzania. VI. Angiosperms (Sapotaceae to Zingiberaceae). Journal of Ethnopharmacology 39, 83–103.

CIA, 2006. The World Fact Book. http://www.cia.gov/cia/publications/factbook/geos/tz.html (accessed on February 27, 2006).

Clark, G.P. 1995. Status Report for 6 Coastal Forests in Lindi Region, Tanzania: 63 pp. Frontier-Tanzania Technical Report No. 18. London & Dar es Salaam, The Society for Environmental Exploration/The University of Dar es Salaam.

de Boer, H.J., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I., Levenfors, J.J., 2005. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. Journal of Ethnopharmacology 96, 461–469.

Dhillion, S.S., Amundsen, C., 2000. In: Svarstad, H., Dhillion, S.S. (Eds.), Responding to Bioprospecting: from Plants in the South to Medicines in the North. Spartacus Forlag, Oslo.

Dhillion, S.S., Gustad, G., 2004. Local management practices influence the viability of the baobab (*Adansonia digitata* Linn.) in different land use types, Cinzana, Mali. Agriculture, Ecosystems and Environment 101, 85–103.

Diallo, D., Paulsen, B.S., 2000. In: Svarstad, H., Dhillion, S.S. (Eds.), Responding to Bioprospecting: from Plants in the South to Medicines in the North. Spartacus Forlag, Oslo.

Eckman, K., Hines, D.A., 1993. Indigenous multipurpose trees in Tanzania. available online http://www.fao.org/docrep/X5327e/x5327e02.htm (accessed on March 13, 2006).

Fyhrquist, P., Mwasumbi, L., Hæggstrøm, C.A., Vuorela, H., Hiltunen, R., Vuorela, P., 2002. Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combretum* (Combretaceae) growing in Tanzania. Journal of Ethnopharmacology 79, 169–177.

Fyhrquist, P., Mwasumbi, L., Hæggstrøm, C.A., Vuorela, H., Hiltunen, R., Vuorela, P., 2004. Antifungal Activity of Selected Species of *Terminalia*, *Pteleopsis* and *Combretum* (Combretaceae) collected in Tanzania. Pharmaceutical Biology 42, 308–317.

Grenier, L., 1998. Working with Indigenous Knowledge. A Guide for Researchers. International Development Research Centre, Ottawa.

GURT (Government of the United Republic of Tanzania), 1998. Tanzania Country Study on Biological Diversity.

Hedberg, I., Hedberg, O., Madati, P.J., Mshigeni, K., Mshiu, E.N., Samuelsson, G., 1982. Inventory of plants used in traditional medicine in Tanzania. I. Plants from the families Acantaceae–Cucurbitaceae. Journal of Ethnopharmacology 6, 29–69.

Hedberg, I., Hedberg, O., Madati, P.J., Mshigeni, K., Mshiu, E.N., Samuelsson, G., 1983a. Inventory of plants used in traditional medicine in Tanzania. II. Dilleniaceae–Opiliaceae. Journal of Ethnopharmacology 8, 105–128.

Hedberg, I., Hedberg, O., Madati, P.J., Mshigeni, K., Mshiu, E.N., Samuelsson, G., 1983b. Inventory of plants used in traditional medicine in Tanzania. III. Papilionaceae–Vitaceae. Journal of Ethnopharmacology 9, 105–128.

IRIN (Integrated Regional Information Network), 2006. http://www.plusnews.org/pnprint.asp?ReportID=2715 (accessed on March 21, 2006).

Iwu, M.M., Laird, S.A., 1998. Drug development and biodiversity conservation in Africa: case study of a benefit-sharing plan. The International Cooperative Biodiversity Groups program (ICBG). http://www.biodiv.org/doc/case-studies/abs/cs-abs-icbg-africa.pdf (accessed on November 1, 2004).

Jäger, A.K., van Staden, J., 2000. The need for cultivation of medicinal plants in southern Africa. Outlook on Agriculture 29, 283–284.

Johns, T., Kokwaro, J.O., Kimanini, E.K., 1990. Herbal remedies of the Luo of Siaya District, Kenya: establishing quantitative criteria for consensus. Economic Botany 44, 369–381.

Johns, T., Mhoro, E.B., Sanya, P., Kimanani, E.K., 1994. Herbal remedies of the Batemi of Ngorongoro District, Tanzania: a quantitative appraisal. Economic Botany 48, 90–95.

Juma, C., 1989. The Gene Hunters. Biotechnology and the Scramble for Seeds. Zed Books Ltd., London.

Kabudi, A.M.M., 1990. Information on indigenous drugs of coastal regions. Tanzania. Research report no. 77, University of Dar es Salaam.

Kabudi, P.J., Svarstad, H., Tvedt, M.W., Bugge, H.C., 2002. Bioprospecting in Tanzania: the question of regulating access to ensure benefit sharing. SUM working paper 2002. 5, University of Oslo, Oslo.

Kauye, C., 2002. A Green Light to FBD. Tanzania Wildlife. No. 27, 15–17.

Kilahama, F.B., 2003. Population Pressure vis-a-vis Conservation of Natural Forests and Woodlands of Tanzania. Tanzania Wildlife No. 29, 44–50.

Kivumbi, C.O., Newmark, W.D., 1991. The history of the half-mile strip on Mount Kilimanjaro. In: Newmark, W.D. (Ed.), The Conservation of Mount Kilimanjaro. IUCN, The World Conservation Union, Nairobi, pp. 81–86.

La Frankie, J.V., 1994. Population dynamics of some tropical trees that yield non-timber forest products. Economic Botany 48, 301–309.

Laird, S.A. (Ed.), 2002. Biodiversity and Traditional Knowledge: Equitable Partnerships in Practice. Earthscan Publications Ltd., London.

Light, M.E., Sparg, S.G., Stafford, G.I., van Staden, J., 2005. Riding the wave: South Africa's contribution to ethnopharmacological research over the last 25 years. Journal of Ethnopharmacology 100, 127–130.

Luoga, E., Witkowski, E.T.F., Balkwill, K., 2000. Differential utilization and ethnobotany of trees in Kitulanghalo forest reserve and surrounding communal lands, Eastern Tanzania. Economic Botany 54, 328–343.

Mahunnah, R.L.A., 2002. Ethnobotany and conservation of medicinal plants in Africa: the way forward in the next decade. In: Proceedings of the 15th Meeting of the Inter-African Experts Committee on African Traditional Medicine and Medicinal Plants, OAU, Lagos.

Marshall, N.T., 1998. Searching for a cure: conservation of medicinal wildlife resources in east and southern Africa. A TRAFFIC Network Report. TRAFFIC International.

Mbwambo, Z.H., Apers, S., Moshi, M.J., Kapingu, M.C., Van Miert, S., Claeys, M., Brun, P., Cos, P., Pieters, L., Vlietinck, A., 2004. Anthranoid compounds with antiprotozoal activity from *Vismia orientalis*. Planta Medica 70, 706–710.

Merker, M., 1904. Die Masai. (Anhang 1), Berlin, 340–349.

Mgumia, F.H., Oba, G., 2003. Potential role of sacred groves in biodiversity conservation in Tanzania. Environmental Conservation 30, 259–265.

Michon, G., De Foresta, H., 1997. Agroforests: pre-domestication of forest trees or true domestication of forest ecosystems? Netherlands Journal of Agricultural Science 45, 451–462.

MNRT (Ministry of Natural Resources and Tourism), 1998. National Forest Policy. Dar es Salaam.

MNRT, 2002. Forest Act, 2002. Dar es Salaam.

MNRT, 2003. Resource economic analysis of catchment forest reserves in Tanzania.

MNRT, 2004. Government Notice No. 160 published on 28/5/2004 (The Forest Regulations, 2004). Dar es Salaam.

Moshi, M.J., Cosam, J.C., Mbwambo, Z.H., Kapingu, M., Nkunya, M.H.H., 2004. Testing byond ethnobotanical claims: brine shrimp lethality of some Tanzanian plants. Pharmaceutical Biology 42, 547–551.

Mukherjee, P.K., Wahile, A., 2006. Integrated approaches towards drug development from ayurveda and other Indian system of medicines. Journal of Ethnopharmacology 103, 25–35.

Nelson-Harrison, S.T., King, S.R., Limbach, C., Jackson, C., Galiwango, A., Kato, S.K., Kanyerezi, B., 2002. Ethnobotanical research into the 21st century. In: Iwu, M.M., Wotton, J.C. (Eds.), Ethnomedicine and Drug Discovery. Elsevier, Amsterdam, pp. 283–306.

Pierce, A.J., Laird, S.A., 2003. In search of comprehensive standards for non-timber forest products in the botanicals trade. International Forestry Review 5, 138–147.

Romero-Daza, N., 2002. Traditional medicine in Africa. The Annals of American Academy of Political and Social Science 583, 173–176.

Ruffo, C.K., 2002. Edible Wild Plants of Tanzania. RELMA/SIDA, Nairobi.

Scheinman, D., The Tanga AIDS Working Group, 1998. A partnership between traditional healers and biomedical personnel. In: Paper distributed at The First National Multisectoral Conference on AIDS, Arusha, TAWG, Tanga.

Schlage, C., Mabula, C., Mahunnah, R.L.A., Heinrich, M., 2000. Medicinal plants of the Washambaa (Tanzania): documentation and ethnopharmacological evaluation. Plant Biology 2, 83–92.

Shrestha, P.M., Dhillion, S.S., 2003. Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. Journal of Ethnopharmacology 86, 81–96.

Smith, W., 1993. Use, abundance and conservation of woody species in the Batemi Valley, North-Western Tanzania. M.Sc. Thesis, Department of Geography, McGill University, Montreal. Journal of Ethnopharmacology 86, 81–96.

Sparg, S.G., Kulcarni, M.G., van Staden, J., 2005. Germination and seedling establishment strategies for *Merwilla natalensis*, a South African plant in high demand. South African Journal of Science 101, 205–208.

Stuart, S.N., Adams, R., Jenkins M. (Eds.) 1990. Biodiversity in Sub-Saharan Africa and its islands: conservation, management and sustainable use. Occasional paper of the IUCN Species Survival Commision. No. 6. IUCN, Gland, Switzerland.

Svarstad, H., Dhillion, S.S. (Eds.), 2000. Responding to Bioprospecting: from biodiversity in the South to medicines in the North. Spartacus Press, AS, Oslo, ISBN 82-430-0163-8, p. 220.

Tabuti, J.R.S., Lye, K.A., Dhillion, S.S., 2003. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. Journal of Ethnopharmacology 88, 19–44.

Tanzania Government Homepage, www.tanzania.go.tz (accessed on February 25, 2006).

ten Kate, K., Laird, S.A., 1999. The Commercial Use of Biodivercity. Earthscan Publications, London.

University of Texas Libraries, 2007. Tanzania Map. www.lib.utexas.edu/maps/cia07/tanzania_sm.2007.gif, accessed 08.02.2008.

Weenen, H., Nkunya, H.H., Bray, D.H., Mwasumbi, L.B., Kilimali, V.A.E.B., 1990. Antimalarial activity of Tanzanian medicinal plants. Planta Medica 56, 368–370.

Paper II

The influence of light and temperature on the germination of two Ugandan medicinal trees

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Abstract

For reasons of the problems of establishment of some Ugandan trees in certain environments, we investigated the influence of temperature and light on germination and seedling growth of two locally threatened medicinal trees, *Hallea rubrostipulata* and *Sarcocephalus latifolius*, to facilitate their establishment. Field and controlled laboratory experiments were carried out to investigate the species germination requirements and seedling growth. Both species needed light to germinate. *Hallea rubrostipulata* had a temperature optimum of 25°C with 79% germination, while for *S. latifolius,* the total germination after 28 days was close to 60% at temperatures from 20 to 35°C. Seedlings of *S. latifolius* survived well at 35°C, while those of *H. rubrostipulata* died at this high temperature. Conversely, seedling of *S. latifolius* died at the low temperature of 15°C. However, in field experiment *S. latifolius* failed to germinate in the available degraded environments, probably because of predation and because the soil is not able to retain water long enough to support seedling growth. We, therefore, conclude that in this part of Uganda, nursery assistance is needed to establish healthy populations of *Sarcocephalus* and many other endangered trees.

Key words: conservation, germination, *Hallea rubrostipulata*, medicinal plants, *Sarcocephalus latifolius*, seedling growth

Résumé

En raison des problèmes que connaissent plusieurs arbres ougandais pour s'établir dans certains environnements, nous avons recherché l'influence de la température et de la lumière sur la germination et la croissance des plantules de deux arbres médicinaux localement menacés, *Hallea rubrostipulata* et *Sarcocephalus latifolius*, afin de faciliter leur établissement. Des expériences de terrain et d'autres contrôlées en laboratoire ont été réalisées afin de découvrir les exigences des espèces pour leur germination et la croissance des plantules. Les deux espèces ont besoin de lumière pour germer. La température optimale pour *H. rubrostipulata* était de 25°C avec 79% de germination, tandis que pour *S. latifolius*, la germination totale était près de 60% après 28 jours à des températures qui allaient de 20 à 35°C. Les jeunes plants de *S. latifolius* survivaient bien à 35°C alors que ceux de *H. rubrostipulata* mouraient à cette haute température. Par contre, les plants de *S. latifolius* mouraient à la basse température de 15°C. Pourtant, sur le terrain, *S. latifolius* n'a pas réussi à germer dans les environnements dégradés qui étaient disponibles, probablement à cause de la prédation et parce que le sol n'était pas à même de retenir l'eau assez longtemps pour permettre la croissance des jeunes plants. Nous en concluons donc que, dans cette partie de l'Ouganda, il faut une aide en pépinière pour établir des populations saines de *Sarcocephalus* et de nombreux autres arbres en danger.

Introduction

Most rural communites in developing countries rely on medicinal plants for their health care (Tabuti, Lye & Dhillion, 2003; Hamilton, 2004). Unfortunately, important medicinal trees are threatened by overexploitation and land use changes (Hamilton, 2004; Kala, Farooqueen & Dhar, 2004). In Uganda, for example, the forest cover has decreased from 13.7% to 3.6% of total land area during the last century (Arinaitwe, Pomeroy & Tushabe, 2000).

Important trees on which local livelihoods depend need to be conserved, which requires a good understanding of their seed and germination ecology (Oryem-Origa, 1999; Peters, 1999; Jäger & van Staden, 2000;

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Oryem-Origa, Kasenene & Magambo, 2004). However, such knowledge on indigenous trees in tropical Africa is very limited.

Many factors, including temperature and light, influence seed germination and seedling establishment. Optimum temperature for germination varies among species. Some have a wide range (Sparg, Kulcarni & van Staden, 2005), while others have a very narrow range (Yirdaw & Leinonen, 2002). The growth rate is the fastest in small seeded species (Fenner & Thompson, 2005; Warren & Adams, 2005; Cardillo & Bernal, 2006). Grime, Mason & Curtis (1981) found that most seeds weighing <0.1 mg were largely photoblastic. Most of positively photoblastic seeds react negatively to the low R : FR ratio of leaf filtered light (Fenner & Thompson, 2005).

In this study, we investigated the effect of temperature and light on germination and seedling growth of two locally important medicinal trees *Hallea rubrostipulata* (K. Schum.) J. F. Leroy (syn. *Mitragyna rubrostipulacea*) and *Sarcocephalus latifolius* (Sm.) E.A. Bruce, (syn. *Nauclea latifolia*) both of the family Rubiaceae. The bark of *H. rubrostipulata* was ranged as the most important treatment against malaria in a recent ethnomedicinal survey in Southern Uganda (Ssegawa & Kasenene, 2007). They found that there is a need to propagate *H. rubrostipulata* in nursery to conserve the natural population in the forest; but repeated germination experiments in two local nurseries at Malabigambo Forest have failed (Eilu, 2007).

Sarcocephalus latifolius is a shrub or small tree in seasonally moist soils of woodland savannas extending from Senegal to Uganda. In the beginning of this study, a focus group discussion with seven traditional healers from Gadumire ranked *S. latifolius* as one of the five most important medicinal plants, but getting difficult to find. The study of Tabuti (2007) confirms that it is overharvested and locally threatened.

Materials and methods

Material

Seeds of *H. rubrostipulata* were collected towards the end of May 2005 from one tree (0°56′60″S, 31°35′19″E; and altitude 1140 m). Each fruit is a capsule about 1-cm long containing numerous minute winged seeds. The species grows in a dense swamp forest in Sango Bay Forest Reserve, Rakai District close to Lake Victoria and near the border to Tanzania. The climax vegetation of the Reserve is evergreen oligotrophic rainforest, previously dominated by *Podocarpus*. The Reserve has a mean annual maximum temperature of 25–27.5°C, a mean annual rainfall of 1300–1500 mm, and there are 90–100 days of rain per year.

Sarcocephalus latifolius fruits were collected in March 2005 from two individual trees growing in disturbed savanna woodland in Kaliro District (1°02′23″N, 33°28′53″E; and altitude 1065 m). The fruit is an irregularly globose berry, 3–8 cm in diameter, containing thousands of minute seeds immersed in a pinkish flesh. Kaliro is situated in East Uganda south of Lake Kyoga. It has a mean annual maximum temperature of 30–32.5°C, mean annual rainfall of 1250–1300 mm, and there are 90–100 days of rain per year.

Seeds were collected from few trees because mature trees with ripe seeds are very rare. The *Hallea* seeds are also difficult to collect as they are 20 m up in the canopy, they easily fall to the ground as they ripen, and are impossible to find on ground because of small size.

Field growth experiment

In March 2005, a field germination experiment with *S. latifolius* was conducted in Gadumire in Kaliro District. Fruits of *S. latifolius* were soaked in water to separate the minute seeds from the dry fruits as recommended by Katende, Birnie & Tengnäs (1995) and then air dried in the shade. Seeds were sown in an agricultural fallow (>2 years) in an area dominated by disturbed wooded savannah. We tested for grazing, shade and different degrees of soil disturbance. The species failed to germinate and the experiment was repeated in November 2005 using the same plots but with different treatments. The experiments were performed in the long (March) and short (November) rainy seasons.

Germination experiment

The minute seeds were stored dry at room temperature for 3 (*H. rubrostipulata*) or 6 months (*S. latifolius*) before sowing. The germination study began in September 2005 and lasted 4 weeks. The average seed weights were 0.00712 mg for *H. rubrostipulata* and 0.0233 mg for *S. latifolius* (n = 50). The seeds were incubated in five controlled environment cabinets at constant temperatures of 15, 20, 25, 30 and 35°C under 12 : 12 h light/dark using Philip master (TDL 36W/830) with photosynthetic

photon flux density (PPFD) at 400–700 nm values approximated 130 μmol m^{-2} s^{-1}.

Five pseudo replicates of 50 seeds of each species were germinated at each temperature in 9-cm Petri dishes on water saturated double filter paper. To prevent evaporation, the Petri dishes were placed in polyethylene bags. Every second day for 28 days, newly germinated seeds were counted and removed to another Petri dish. Germination was defined as radicle emergence. To determine the effect of continuous darkness, five pseudo replicates of each species were wrapped in aluminium foil and incubated in the same cabinets. The seeds under dark treatment were counted under safe green light. Distilled water was added regularly to the dishes as needed.

Seedling growth experiment

The study of seedling growth started in mid-January 2006 and lasted 12 weeks. Seeds were sown on saturated filter paper in 9-cm Petri dishes and placed in a controlled environment cabinet at 25°C. After germination, when the seedlings had unfolded the two cotyledons, the seedlings were transferred to pots (8 cm) using a standard potting compost mixed with perlite and placed in five growth chambers at constant temperatures of 15, 20, 25, 30 and 35°C under 12 : 12 h light/dark. Light sources were Osram Powerstar HQI-BT 400W, Osram, Munich, Germany supplemented by incandescent lamps to lower the R : FR ratio. PPFD values were 200 μmol m^{-2} s^{-1} (light to medium shade), R : FR 1.6 and relative humidity 90%.

Five seedlings of each species were harvested, dried and weighed at the time of planting (time 0). After week 4, four seedlings of *S. latifolius* and five of *H. rubrostipulata* plants from each temperature were harvested, separated into shoots and roots, dried at 70°C for 48 h and then weighed. Harvesting continued every second week until the twelfth week after planting.

Relative growth rate and statistical analysis

Relative growth rates (RGR) at the different temperatures and at different phases of seedling growth were determined by taking the natural logarithm of change in dry mass (W) of shoots or roots to change in time (t) as follows (Hunt *et al.*, 2002):

$$\mathrm{RGR} = \ln\left(\frac{W_2 - W_1}{t_2 - t_1}\right).$$

One-way ANOVA test for percentage germination versus temperature was run for both species. Two-way ANOVA was used to test if germination was significantly different between the two species. The Minitab statistical program was used for the tests.

Results

Germination

In the field experiments, no seeds of *S. latifolius* were observed to germinate. However, during the laboratory experiment, close to 60% of the same batch of seeds germinated at 20, 25, 30 and 35°C (Fig. 1). Germination was completed within 15 days at the temperatures 25 and 30° C. Total germination took more days (25) at 20 and 35 °C (Fig. 2). At 15 °C, no seeds germinated.

Hallea rubrostipulata had optimum germination (78.8%) at 25°C (Fig. 1). At 20 and 30 °C, germination were 59.6 and 67.2°C respectively. Most of the seeds had germinated within 6 days and germination was completed by the ninth day at 25 and 30°C (Fig. 2). No seeds of *H. rubrostipulata* germinated at 35°C. At the low temperature of 15°C, the capability of *H. rubrostipulata* to germinate was low (25%) and delayed.

For both species, very few seeds germinated in the dark (0.8% of *Hallea* at 20 and 25°C; 5.6% and 4% of *Sarcocephalus* at 20 and 25°C respectively).

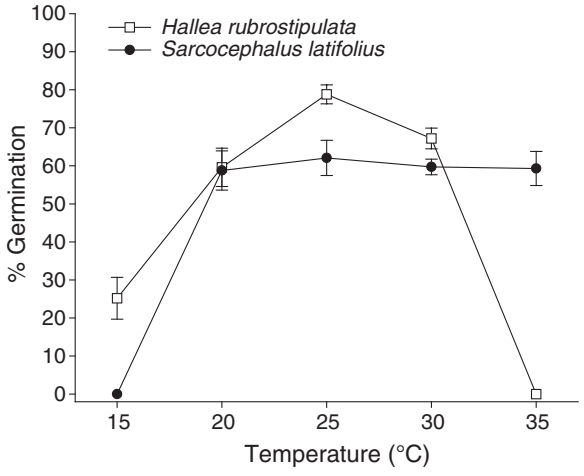

Fig 1 Temperature effect on total per cent germination after 28 days of *Hallea rubrostipulata* and *Sarcocephalus latifolius* sown at five temperatures (15, 20, 25, 30 and 35°C) and 12 : 12 h light/dark. Bars show standard errors of the mean (n = 5)

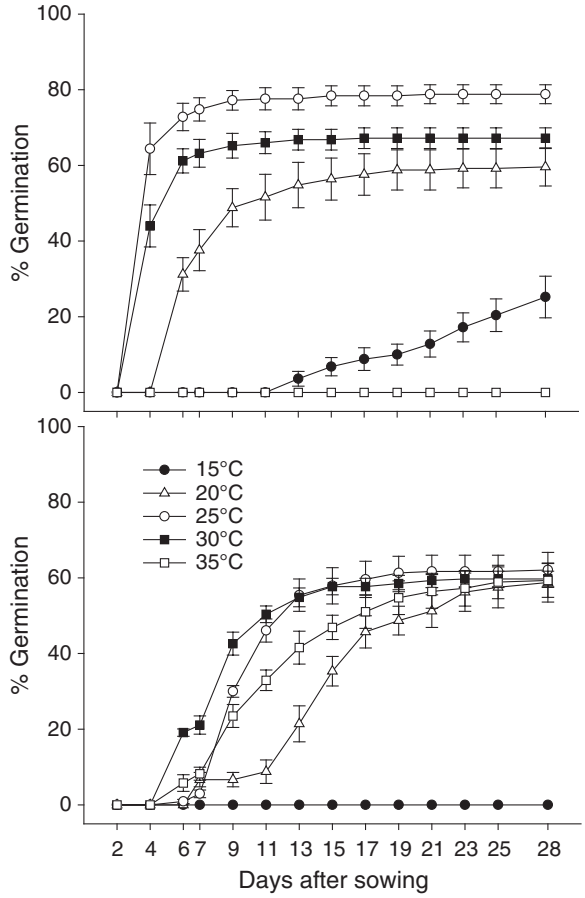

Fig 2 Cumulative per cent germination of *Hallea rubrostipulata* (above) and *Sarcocephalus latifolius* (below) sown in Petri dishes and incubated at five constant temperatures and 12 : 12 h light/dark for 28 days. Bars show standard errors of the mean (n = 5)

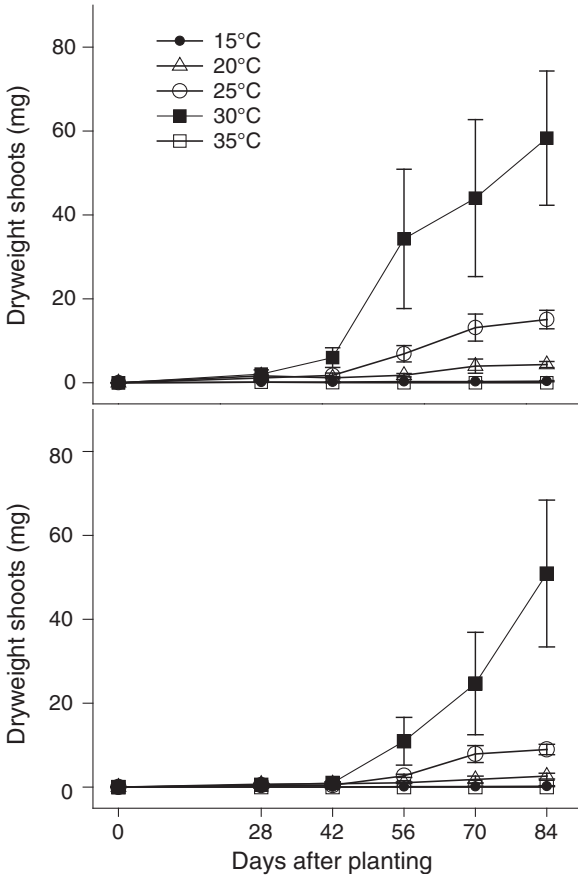

Fig 3 Dry weight of shoots and roots of *Hallea rubrostipulata* grown in pots at five constant temperatures and 12 : 12 h light/dark and harvested every second week from week 4 to week12 after planting (n = 5). Bars show standard errors of the mean

Seedling growth

Both species had the best growth at 30°C, and this was increasing linearly with time (Figs 3 and 4). For *S. latifolius*, the growth in terms of dry weight of the shoots was almost twice as high as the dry weight of roots, while for *H. rubrostipulata* dry weight of roots and shoots was almost similar. After 12 weeks, dry weight of *Sarcocephalus* roots and shoots were almost ten times higher than that of *Hallea. Sarcocephalus latifolius* had its second best growth at 35°C, while *Hallea* died at this high temperature.

In both species, RGR was the highest and showed the clearest trends in the first 4 weeks (Fig. 5, Table 1). *Sarcocephalus latifolius* showed a linear increase with temperature between the temperatures of 15 and 30°C in both root and shoot. *Hallea* had a somewhat lower and broader optimum of RGR between 25 and 30°C (Fig. 5). For the periods after 42 days, the RGR of *S. latifolius* declined, while for *H. rubrostipulata* it still increased for 14 days and then declined (Table 1).

After 9 months in greenhouse at the same environment, the two species had about the same height of 50 cm.

Two-way ANOVA for per cent germination versus species and temperature showed that there was effect of species on some of the temperatures ($F = 39.14$, $P < 0.001$).

Discussion

As *S. latifolius* seeds failed to germinate under disturbed conditions in wooded savanna, constraints like water stress and herbivory could have affected germination and

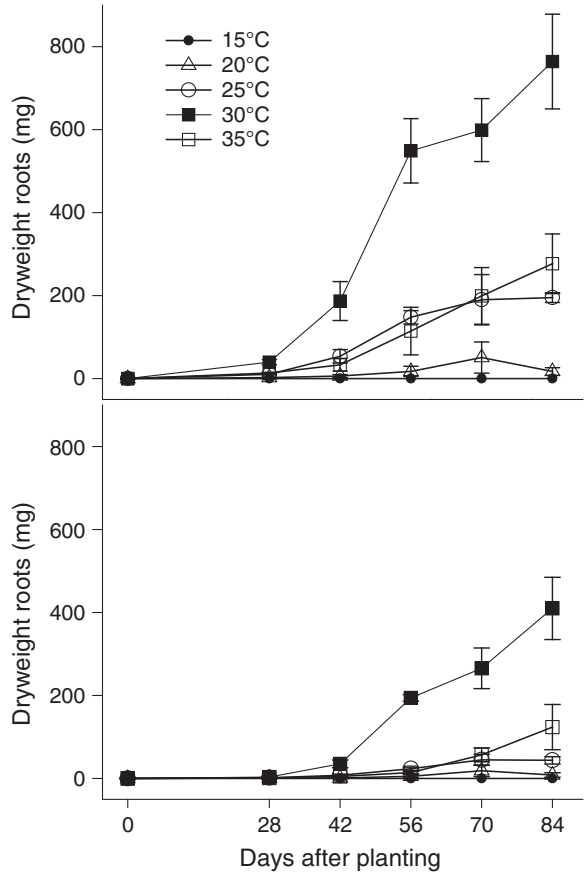

Fig 4 Dry weight of shoots and roots of *Sarcocephalus latifolius* grown in pots at five constant temperatures and 12 : 12 h light/dark and harvested every second week from week 4 to week 12 after planting (n = 4). Bars show standard errors of the mean

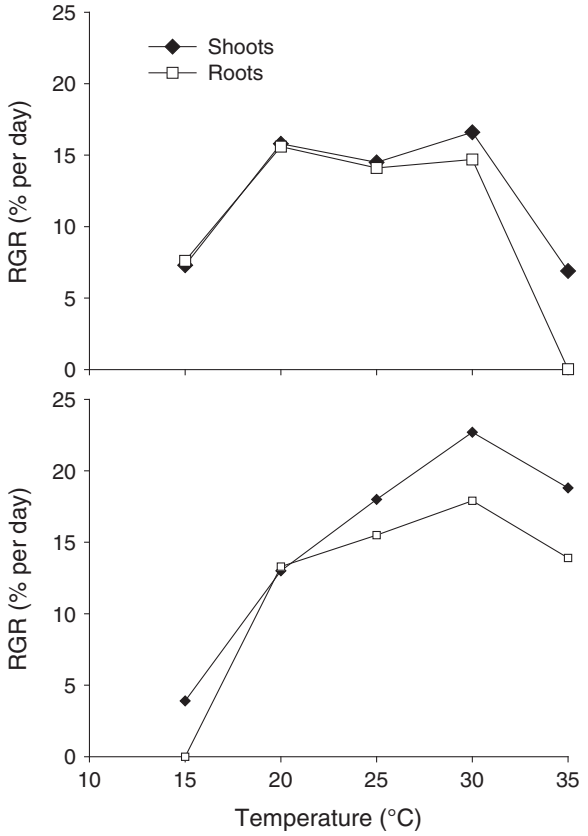

Fig 5 Relative growth rate (RGR) mg g^{-1} day^{-1} of *Hallea rubrostipulata* (above) and *Sarcocephalus latifolius* (below) during the first 28 days of the experiment

survival. As termites were abundant in and around the experimental field, they may have eaten or collected the small seeds of *S. latifolius*. The shaded plots were more protected against drought and sunburn, but lack of germination here could be because of low R : FR (Grime *et al.*, 1981; Yirdaw & Leinonen, 2002). This implies that the species cannot be established easily without nursery assistance.

As it is well known that species with seeds weighing <0.1 mg usually require light to germinate (Grime *et al.*, 1981; Fenner & Thompson, 2005), it was not unexpected that few seeds of *S. latifolius* and *H. rubrostipulata* germinated in darkness (0.8% of *Hallea* at 20 and 25°C; 5.6% and 4% of *Sarcocephalus* at 20 and 25°C respectively).

Although both species overlapped in terms of their preferred temperature for germination and early seedling establishment (20–30°C), *S. latifolius* had a higher temperature range than *H. rubrostipulata* and this reflected their natural ecological adaptations. The natural habitat where *S. latifolius* grows in Uganda has higher temperature ranges than those of Sango Bay Forest Reserve according to Atlas of Uganda (Anonymous 1967). *Hallea rubrostipulata* managed to establish at the lower temperature of 15°C, while *S. latifolius* failed. Simon *et al.* (1976) found that germination of tropical species declines dramatically at about 14°C and ceases at 10°C. This is consistent with our findings for *H. rubrostipulata,* but not with our findings for *S. latifolius* where the lower germination limit was between 15 and 20°C. The two tree species thus show an amazing adaptation of seed germination and early seedling growth to their prevailing environmental conditions.

As *Hallea* germinated after 4 days and many seeds were contaminated by fungi within 4 days, especially at 20°C, we conclude that rapid germination may be a way of

Table 1 Relative growth rate (RGR) of shoots and roots of *Hallea rubrostipulata* (*H*) and *Sarcocephalus latifolius* (*S*) grown at five temperatures and harvested five times from 28th to 84th day after planting

		RGR[b] (mg g^{-1} day^{-1})			
Treatment (days)[a]	Temp. (°C)	Shoots *H.*	Shoots *S.*	Roots *H.*	Roots *S.*
$T_2 - T_1$ (28 − 0)	35	69	188	25	139
	30	166	227	148	179
	25	145	180	141	155
	20	158	130	156	133
	15	74	39	8	−8
$T_3 - T_2$ (42 − 28)	35	−73	76	–[b]	118
	30	88	129	51	189
	25	35	136	1	116
	20	−29	72	11	21
	15	12	−169	−22	−92
$T_4 - T_3$ (56 − 42)	35	–[b]	103	–[b]	87
	30	145	90	197	142
	25	114	84	146	93
	20	35	86	21	116
	15	36	–[b]	53	–[b]
$T_5 - T_4$ (70 − 56)	35	–[b]	46	–[b]	115
	30	21	7	68	26
	25	54	21	89	54
	20	66	89	45	103
	15	2	–[b]	24	–[b]
$T_6 - T_5$ (84 − 70)	35	–[b]	27	–[b]	65
	30	23	20	60	36
	25	11	2	11	2
	20	7	90	30	−66
	15	29	–[b]	38	–[b]

[a]T is number of days from T_1: the day of potting.
[b]No data indicate that the plants have died.

escaping mortality by fungi, as suggested by Muhanguzi, Obua & Oryem-Origa (2002) for some Ugandan forest trees. The *Sarcocephalus* seeds germinated slightly later and were more resistant to fungal infection (about 50% of the seeds had germinated within 10 days at the same temperatures).

There were striking differences between the species in biomass allocation to shoots and roots. While at the end of the experiment, *Hallea* had almost as much biomass in roots as in shoots, *Sarcocephalus* had only about half as much biomass in the roots as in the shoots. The slow growth of the *Hallea* seedlings and the relatively large allocation to roots might be a strategy for adapting to an oligotrophic environment.

The high germination percentage (60% and 60–80%) after 3 or 6 months storage indicates that seeds of these species can be stored for considerable time, but the actual length of seed longevity must be studied further.

The light treatment given was suitable for seedling growth (200 μmol m^{-2} s^{-1}, R : FR = 1.6), which corresponds to light to medium-shade (Lee *et al.*, 1996, 1997; full sun: 1528 μmol m^{-2} s^{-1}, R : FR = 1.34; light shade: 400–600 μmol m^{-2} s^{-1}, understory shade: 13 μmol m^{-2} s^{-1}, R : FR = 0.20). Several studies have given the best seedling growth at moderate shade and high R : FR (Lee *et al.*, 1996; Yirdaw & Leinonen, 2002). Moderate shade results in better survival during the dry season (McLaren & McDonald, 2003; Lemenih, Gidyelew & Teketay, 2004).

To domesticate *Hallea* and *Sarcocephalus* successfully, it will be important to find or create habitats with the preferred light and temperature requirements of the species. Our field experiment indicates that natural establishment of *S. latifolius* in degraded environment is very difficult without nursery assistance. For *Hallea*, repeated germination experiments in the local nurseries at Malabigambo Forest also failed.

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References

Anonymous (1967) *Atlas of Uganda*. Department of Lands and Survey, Entebbe, Uganda.

Arinaitwe, H., Pomeroy, D. & Tushabe, H., Eds (2000) *The State of Uganda's Biodiversity 2000*. National Biodiversity Data Bank, Makerere University, Kampala.

Cardillo, E. & Bernal, C.J. (2006) Morphological response and growth of oak *(Quercus suber* L.) seedlings at different shade levels. *For. Ecol. Manage.* **222**, 296–301.

Eilu, G., Ssegawa, P., Obua, J. & Galabuzi, C. (2007) Progress report for the period October 2006 to March 2007. Available at:

http://www.plantlife.org.uk/international/plantlife-med-plants-projects-allachy-uganda.htm (accessed on 19.11.2007).

FENNER, M. & THOMPSON, K. (2005) *The Ecology of Seeds*. University Press, Cambridge.

GRIME, J.P., MASON, G. & CURTIS, A. (1981) A comparative study of germination characteristics in a local flora. *J. Ecol.* **69,** 1017–1059.

HAMILTON, A. (2004) Medicinal plants, conservation and livelihoods. *Biodivers. Conserv.* **13,** 1477–1517.

HUNT, R., CAUSTON, D.R., SHIPLEY, B. & ASKEW, A.P. (2002) A modern tool for classical plant growth analysis. *Ann. Bot.* **90,** 486–488.

JÄGER, A.K. & VAN STADEN, J. (2000) The need for cultivation of medicinal plants in Southern Africa. *Outlook Agric.* **29,** 283–284.

KALA, C.P., FAROOQUEEN, N.A. & DHAR, U. (2004) Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India. *Biodivers. Conserv.* **13,** 453–469.

KATENDE, A.B., BIRNIE, A. & TENGNÄS, B. (1995) *Useful Trees and Shrubs for Uganda*. Technical Handbook No 10. Reg. Soil Conserv. Unit., Nairobi.

LEE, D.W., BASKARAN, K., MANSOR, M., MOHAMAD, H. & YAP, S.K. (1996) Irradiance and spectral quality affect Asian tropical rain forest tree seedling development. *Ecology* **77,** 568–580.

LEE, D.W., OBERBAUER, S.F., BASKARAN, K., MANSOR, M., MOHAMAD, H. & YAP, S.K. (1997) Effects of irradiance and spectral quality on seedling development of two South Asian *Hopea* species. *Oecologia* **110,** 1–9.

LEMENIH, M., GIDYELEW, T. & TEKETAY, D. (2004) Effects of canopy cover and understory environment of tree plantations on richness, density and size of colonizing woody species in southern Ethiopia. *For. Ecol. Manage.* **194,** 1–10.

MCLAREN, K.P. & MCDONALD, M.A. (2003) The effect of moisture and seedling germination and seedling survival in a tropical dry forest in Jamaica. *For. Ecol. Manage.* **183,** 61–75.

MUHANGUZI, H.D.R., OBUA, J. & ORYEM-ORIGA, H. (2002) Influence of light quality on the germination characteristics of seeds of selected pioneer, under storey and canopy tree species in Kalinzu forest reserve, Uganda. *Ug. J. Agric. Sci.* **7,** 25–30.

ORYEM-ORIGA, H. (1999) Fruit and seed ecology of wild robusta coffee (Coffea Canephora Froener) in Kibale National park, Uganda. *Afr. J. Ecol.* **37,** 439–448.

ORYEM-ORIGA, H., KASENENE, J.M. & MAGAMBO, J.S. (2004) Some aspects of wild robusta coffee seedling growth in Kibale National Park, Uganda. *Afr. J. Ecol.* **42**(Suppl. 1), 34–39.

PETERS, C.M. (1999) Ecological research for sustainable non-wood forest product exploitation: an overview. In: *Non-wood Forest Products of Central Africa: Current Research Issues and Prospects for Conservation and Development* (Eds T. C. H. SUNDERLAND, L. E. CLARK and P. VANTOMME). Food and Agriculture Organization, Rome.

SIMON, E.W., MINCHIN, A., MCMENAMIN, M.M. & SMITH, J.M. (1976) The low temperature limit for seed germination. *New Phytol.* **77,** 301–311.

SPARG, S.G., KULCARNI, M.G. & VAN STADEN, J. (2005) Germination and seedling establishment strategies for *Merwilla natalensis,* a South African plant in high demand. *S. Afr. J. Sci.* **101,** 205–208.

SSEGAWA, P. & KASENENE, J.M. (2007) Plants for malaria treatment in Southern Uganda: traditional use, preference and ecological viability. *J. Ethnobiol.* **27,** 110–131.

TABUTI, J.R.S. (2007) The uses, local perceptions and ecological status of 16 woody species of Gadumire Sub-county, Uganda. *Biodivers. Conserv.* **16,** 1901–1916.

TABUTI, J.R.S., LYE, K.A. & DHILLION, S.S. (2003) Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. *J. Ethnopharmacol.* **88,** 19–44.

WARREN, C.R. & ADAMS, M.A. (2005) What determines interspecific variation in relative growth rate of *Eucalytus* seedlings? *Ecophysiology* **144,** 373–381.

YIRDAW, E. & LEINONEN, K. (2002) Seed germination responses of four afromontane tree species to red/far-red ratio and temperature. *For. Ecol. Manage.* **168,** 53–61.

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Paper III

The framework tree species approach to conserve medicinal trees in Uganda

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Abstract

Framework species are indigenous tree species planted in a mixed stand to accelerate natural regeneration of forest and encourage biodiversity regeneration. In this study we used the framework species method to make multipurpose tree gardens to provide traditional healers with woody species used for medicine and other needs like food and firewood. We specifically determined the phenology, germination behaviour, survival and growth after planting 27 mainly indigenous woody species. The species were planted in a mixed stand together at a density of 3125 ha^{-1}. Field performance was assessed by monitoring survival, height and crown width once every month for 13 months after planting. Eleven species (*Artocarpus heterophyllus, Calliandra calothyrsus, Callistemon citrinus*, *Carica papaya, Carissa spinarum, Leucaena leucocephala, Markhamia lutea, Sarcocephalus latifolius, Senna siamea, S. spectabilis* and *Terminalia schimperiana*) proved to be excellent framework species. Eight species qualified as 'acceptable' FWS (*Albizia coriaria, Ceiba pentranta, Entada abyssinica, Erythrina abyssinica, Eugenia jambos, Ficus sycomorus, Maesopsis eminii* and *Milicia excelsa),* while seven species were ranked as 'marginally acceptable' (*Acacia macrothyrsa, Calpurnia aurea, Canarium schweinfurthii, Capparis tomentosa, Ficus natalensis, Senna sp.* and *Warburgia salutaris). Annona squamosa* was the only species rejected since both germination and survival was low. Trees with good reaforestation traits could be recommended for planting while the species that were marginally acceptable or rejected require extra research since some of them are important medicinal woody species of conservation concern.

Key words: framework species, medicinal woody species, seedling performance, reaforestation

Introduction

The dry forest in Africa is one of the most degraded forests in the world, and often densely populated. Most of the rural population is poor and depend on forest resources for their livelihood. Non-timber forest products (NTFP) have been harvested by people for subsistence use and trade for thousands of years. Population increase and growing commercial trade of NTFPs, especially medicinal plants, have caused increasing concerns on the sustainability of their harvesting (Hamilton 2004; Ticktin 2004). It is estimated that up to 15000 medicinal and aromatic plant species are threatened with extinction worldwide. Woody species are especially threatened because they are slow growing (Hawkins 2008; Schippmann et al. 2002) and often roots and bark are used.

Medicinal plants are very important because they contribute to primary health care by providing an easily accessible and affordable source of medication. It is estimated that in Uganda, 70-80 % of the population use medicinal plants for their primary health care. Medicinal plants contribute over 57% to the forest revenue in Madagascar (Walter 2001) and income in foreign earnings for Namibia from the sales of Devil's Claw (*Harpagophytum procumbens*) in 2002 was estimated at US$ 2.7 million (Cole 2003). Besides their medicinal values woody species also have other values such as provision of firewood. In Uganda, 90% of the population depends on firewood for cooking, and about 1 million earn their income from forest products (NEMA 2002).

Considering the important values that these species have, it is important that they are conserved to minimise the suffering of the marginalised who cannot afford expensive allopathic medicines, and to reduce poverty levels of practitioners and other people who depend on medicinal plants for income generation.

One conservation strategy is cultivation of these species. Cultivation has several advantages, for instance, adulteration of species is easily avoided, postharvest handling can be controlled and a steady source of uniform quality raw material can be secured (Schippmann et al. 2002). On the other hand it is possible that the content of secondary metabolites will be different for cultivated species.

In this paper we describe how we applied a modified version of the framework species (FWS) method (Blakesley et al. 2002; Elliott et al. 2003; Elliott et al. 2008) to make multipurpose tree gardens in Kaliro District, Uganda. Seedlings of mainly local tree species were raised in a

nursery and planted in one single event. Species that satisfy the FWS should have i) a rapid and even germination, ii) high field performance (high survival and rapid growth), iii) dense and spreading crown that outcompete weeds and iv) provide resources that attract seed-dispersing wildlife (fruits, nectar, nesting sites etc.). The FWS method was developed to restore forest and has proved very successful in Queensland, Australia (Tucker and Murphy 1997) where framework species plots were reported to be colonised by 15-49 naturally establishing trees within 5-7 years of planting, and in Thailand (Blakesley et al. 2002; Elliott et al. 2003). We planted seedlings of 27 woody species out of which 10 species (*Acacia macrothyrsa, Albizia coriaria, Calpurnia aurea, Capparis tomentosa, Carissa spinarum, Entada abyssinica, Erythrina abyssinica, Markhamia lutea, Sarcocephalus latifolius* and *Warburgia salutaris*) are highly preferred medicinal plants in Uganda and elsewhere (Kokwaro 1976; Heine and Heine 1988; Chabra et al. 1987; Adjanohoun et al. 1993; Neuwinger 1996; Geissler et al. 2002; Tabuti et al. 2003; Krog et al. 2006; Kisangau et al. 2007; Ssegawa and Kasenene 2007; Tabuti 2007; Lye et al. 2008; van Wyk 2008; Tabuti et al. 2009).

The specific objectives of this study were i) to gain knowledge about flowering and fruiting phenology and seed germination of selected medicinal trees, ii) to assess each species as potential framework species and iii) to find out if the framework species can be successfully used for forest restoration or establishment of community forests in Uganda.

Material and methods

Study site

Field gardens were established in the sub-counties of Gadumire and Nawaikoke in Kaliro District. Kaliro District is situated 200 km northeast of Kampala, 33º30´-33 º35´ E and 1º04´-1º15´ N at an elevation of 1030-1080 m above sea level. The district has two rainy seasons, March-May and August-September. The mean annual rainfall is 1250-1300 mm and the mean annual maximum temperature is 30-32.5ºC. The district is heavily inhabited with a population density in excess of 280 people per square kilometre. Much of the original vegetation has been destroyed and the landscape converted to small scale farmland. The district is dominated by small scale farms and papyrus swamps.

Methology

Phenology

We observed the flowering and fruiting of *Capparis tomentosa* (n= 5)*, Psorospermum febrifugum* (n= 35)*, Sarcocephalus latifolius* (n= 3) and *Securidaca longipedunculata* (n= 27) in Nawaikoke every second week from November 2006 to November 2007; *Hallea rubrostipulata* (n=30) and *Warburgia salutaris* (n= 6) from May 2006 to November 2007 in a tropical wet forest reserve in Sango Bay in south-western Uganda. One of the *W. salutaris* trees died during the time of monitoring due to severe debarking. The trees were given scores from 0 to 5 for flowering and fruiting stages (Okullo et al., 2004). Zero was scored for individuals where no flowering or fruiting was observed and 5 for withering flowers and end of fruiting (75% of fruits harvested or fallen).

Seed collection and germination

We used a slightly modified FWS method, where we tested for germination traits as described by Blakesley et al. (2002), but without using a replicated experimental design. We raised seedlings for 4 to 12 months, planted the seedlings in three plots in Kaliro district and monitored them for survival and growth broadly as described by Elliott et al. (2003) although we monitored for 13 months instead of 17 and we did not monitor weed cover. The tree seedlings were raised in a nursery established for this purpose in Nawaikoke from March 2007 to April 2008. Most of the seeds were collected in the neighbouring farms and fallows.

The seeds were cleaned and between 20 and 200 seeds of each species were sown in polyethylene pots or germination trays and placed under shade net that removed 70% of the light. Some very small seeds, like those of *Sarcocephalus latifolius* and *Milicia excelsa* were sown in greater number and directly in seedbeds. The seeds were not pre-treated and were sown in a medium of 50/50% mixture of river sand and forest soil. We added two introduced species (*Calliandra calothyrsus* and *Leucaena leucocephala)* which fix nitrogen and improve soil quality, and some *Senna* species which grow fast and are wanted by the population for firewood (Tabuti et al. 2009). All the species are growing in the area and have proved not to be invasive.

Germination was recorded every third day. As the seedlings grew they were pricked out and transplanted to bigger polyethylene pots. The transplanting medium contained less sand, and

some composted cow dung was added. The plants were watered when necessary in the dry season. The seedlings were hardened by exposing them to gradually more sun and less watering in the last one to two months before planting.

Planting and monitoring of growth

Three groups of Traditional Healers provided land and management of the seedlings after planting. One healer in each group arranged to have a medicinal tree garden on their own land. After a meeting and discussion we developed a written agreement, describing rights and responsibility of each party. Most importantly we agreed that the trees were not to be harvested during the first year while we monitored growth, but that afterwards the healers could harvest as they pleased. We provided seedlings and money for ploughing and fence material, while the groups of healers prepared land, put up the fence, planted the seedlings and weeded the plots three times during the first rainy season.

In March 2008 three experimental plots were selected, two in Nawaikoke and one in Gadumire. Each plot measuring 40 x 40 m and was prepared as described above. Holes (0.5 m in diameter and 0.5 m deep) for planting were dug and seedlings transported to the plots just before the rainy season.

Two weeks after the rain had started a mixture of 27 indigenous and some introduced seedlings (*Calliandra calothyrsus* and *Leucaena leucocephala)* were randomly planted in each plot. Depending on seedling availability, we planted from 4 to 30 seedlings of each species in each plot at a spacing of 1.8 m. Seedlings of ten more species were planted, but because less than 3 seedlings were available for each plot, we decided to not include them in this paper. Watering was only done during planting. Beans were planted between the lines during the first rainy season to provide some short term benefit and increase motivation for weeding. This also resulted in improved soil fertility by nitrogen fixation.

Sub-samples of seedlings (maximum ten of each species in every plot, 813 trees altogether) were randomly selected and labelled for subsequent monitoring. All labelled trees were monitored after two weeks and thereafter once every month for height, crown width, root collar diameter and health. Health was recorded on a score of 0-3 (0: dead; 3: completely healthy).

To assess the species' performance as framework species, we used the criteria advocated by Elliott et al. (2003), but modified their methods by monitoring growth for a shorter period of time. In addition the rainfall in Elliot's area was much higher than in Kaliro (2095 mm and 1275 mm respectively). According to Elliott et al. (2003), potential framework species should be assessed for germination percentage, mean length of dormancy (MLD), synchrony of germination (number of days from the first to the last seed germinated) and seedling survival, height and crown width after planting in the field. Germination is regarded to be good if 60% or more of the seeds germinate and low if less than 20% germinate. The MLD, i.e. the number of days when 50% of the seeds have germinated, is considered to be rapid if seeds take 21 days or less to germinate and slow if they take 84 days or more. Germination is defined as synchronous if all seedlings of a species emerge within 21 days, and highly asynchronous if they take more than 84 days (Blakesley et al., 2002).

The survival rate is considered to be excellent when 70% or more seedlings of any species survive after 17 months; acceptable when 50 to 69% survive, and marginally acceptable when 45-49% of the seedlings survive.

The crown width was monitored as an additional measure of growth and to establish the species' ability to form a closed canopy. A closed canopy will suppress weeds and create a cooler, more shady and moist environment, where forest tree seedlings can establish more easily. A crown width of 1.4 m after 13 months was considered as excellent, 1-1.39 m as acceptable and less than 0.6 m as not acceptable. We considered a mean height of 1.6 m after 13 months as excellent, 1-1.59 m as acceptable and 0.6-0.99 m mean height as marginally acceptable for woody species in this dry area.

The number of saplings of each species monitored 13 months after planting ranged from 3 to 29. This variation in number was due to variation in availability of seeds/seedlings, seedling mortality in the nursery prior to planting and survival in the plots (Table 2).

Results

Phenology

Sarcocephalus latifolius portrayed a bi-modal fruiting pattern, producing fruits during December, and from February to August. A similar trend was observed for *Warburgia salutaris*, where fruits were produced in September and March. *Securidaca longipedunculata* had produced seeds in November-December during the first year of monitoring, but in the subsequent year fruits did not develop as the flowers were destroyed by a hailstorm in May. *Psorospermum febrifugum* only had one long fruiting period of seven months (Table 1). *Capparis tomentosa* did not produce flowers or fruits during the time of observation.

Germination

Germination ranged from 7 to 100% among the 27 species of mainly indigenous trees and shrubs in the experiment (Table 2). Nearly half of the species (*Artocarpus heterophyllus, Calliandra calothyrsus, Callistemon citrinus, Canarium schweinfurthii, Capparis tomentosa, Carica papaya, Carissa spinarum, Erythrina abyssinica, Eugenia jambos, Leucaena leucocephala, Maesopsis eminii, Markhamia lutea, Milicia excelsa* and *Sarcocephalus latifolius*) had a germination of 60% or higher which is regarded as good for this kind of nursery production. Five of the species (*Acacia macrothyrsa, Ceiba pentandra, Ficus sycomorus, Calpurnia aurea* and *Senna grandis*) had low germination of less than 20%. For dry forest species like this, even less than 20% germination can be acceptable. Since we wanted to explore the germination traits of the different seeds, we did not pretreat them. Seeds of both *Capparis tomentosa* and *Warburgia salutaris* are known to lose viability quickly after harvesting. Seeds of *C. tomentosa* were sown immediately after collection and achieved 100% germination while seeds of *W. salutaris* were not sown until two weeks after collection (for logistical reasons) and only 32% germinated.

Mean length of germination across all species varied between 8 – 50 days and synchrony of germination varied between 1-93 days (Table 3). Thirteen of the species had rapid germination ($\leq$ 21 days), only one, *Senna grandis,* had asynchronous germination while the rest had intermediate germination (between 21-84 days).

Survival

Of the 27 species of trees planted, 18 or 67% had excellent survival rates ranging between 72-100% 13 months after planting (Table 2); these species are *Albizia coriaria, Artocarpus heterophyllus, Calliandra calothyrsus, Callistemon citrinus, Capparis tomentosa*, *Carica papaya, Ceiba pentantra, Carissa spinarum, Erythrina abyssinica, Eugenia jambos, Ficus sycomorus, Leucaena leucocephala, Markhamia lutea, Milicia excelsa, Sarcocephalus latifolius, Senna siamea, S. spectabilis* and *Terminalia schimperiana*. Seven species had unacceptable survival rates between 13 and 42%.

Growth

Nine of the species planted *(Calliandra calothyrsus, Callistemon citrinus, Carica papaya, Ceiba pentandra, Ficus sycomorus, Leucaena leucocephala, Sarcocephalus latifolius, Senna siamea* and *S. spectabilis)* showed excellent growth of 1.6 m or higher (Table 4), eight displayed acceptable height of 1 m or more, four species had marginally acceptable growth of 0.6 to 0.99 cm, and six species had unacceptable growth of less than 0.59 m

Crown width

Nine of the species had excellent crown width of 1.4 m or more 13 months after planting, only four had acceptable crown width of 1 to 1.39 m; while eleven had marginal (0.6-0.99 m) and three had unacceptable crown width of less than 0.6 m (Table 4).

Discussion

This study quantifies for the first time germination traits and field performance of 27 woody species growing in Uganda. Botanic Gardens Conservation International regards five of these species to be medicinal trees of conservation concern (*Albizia coriaria, Carissa spinarum, Erythrina abyssinica, Markhamia lutea,* and *Warburgia salutaris;* Hawkins 2008). Another two species, *Sarcocephalus latifolius* and *Markhamia lutea* are very rare or rare and decreasing in Nawaikoke, Uganda (Tabuti et al. 2009). *Capparis tomentosa* was the top priority for a group of healers in Gadumire when selecting important medicinal plants that are becoming increasingly

difficult to find. *C. spinarum* and *S. latifolius* are both important medicinal plants, but harvesting practices have been detrimental since few trees are left and mostly bark and roots are used (Tabuti et al. 2009).

Phenology studies are important during domestication of woody species because they facilitate seed collection and give information on the best sowing procedures. Phenology data on five important medicinal woody species are presented here for the first time. The low number observed for three of them (*C. Tomentosa, S. latifoilius* and *W. Salutaris)* reflects how rare they are, no more individuals were found in the areas studied. We did not observe flowering of *S. latifolius* probably due to a very ephemeral flowering phenophase, but during the time of monitoring we recorded two fruiting periods for both *S. latifolius* and *W. salutaris*. Germination success, mean length of dormancy and synchrony of germination are regarded as important traits for species raised in nurseries for use in tree planting and reforestation. In this study 48% of the trees studied had good germination (higher than 60%). This contrasted with observations by Elliott et al. (2002), where 80% of the species had good germination. African tree species may thus have lower germination success or greater need of pre-treatment than Asian species. Further work should focus on establishing seed pre-treatments that improve germination. Several *Acacia* and *Senna* species have thick seed coats and achieve higher germination success when pre-treated (Katende et al. 1995). Nevertheless, survival was satisfactory as 63% species planted had an excellent survival rate of 70% or more, even though Kaliro district suffered a severe drought in 2009, and the rains did not start until the beginning of April. This is in line with Elliott et al. (2002) where they planted seedlings in two succeeding years. Of the seedlings planted in 1998 eighty % had an excellent survival, while for the seedlings planted in 1999 only 28% of the species had excellent survival, probably due to dry weather after planting.

Eleven of the twenty seven tree species planted (*Artocarpus heterophyllus, Calliandra calothyrsus, Callistemon citrinus*, *Carica papaya, Carissa spinarum, Leucaena leucocephala, Markhamia lutea, Sarcocephalus latifolius, Senna siamea, S. spectabilis* and *Terminalia schimperiana*) proved to be excellent framework species (Table 3). Eight species qualified as 'acceptable' FWS (*Albizia coriaria, Ceiba pentranta, Entada abyssinica, Erythrina abyssinica, Eugenia jambos, Ficus sycomorus, Maesopsis eminii* and *Milicia excelsa),* while six species were ranked as 'marginally acceptable' (*Acacia macrothyrsa, Canarium schweinfurthii,*

Capparis tomentosa, Ficus natalensis, Senna sp. and *Warburgia salutaris). Annona squamosa* was the only species rejected since both germination and survival was low.

We started to raise seedlings one year before planting, but we experienced that this was early. Probably we needed between three and six months to raise many of the species, as they were growing rapidly in nursery, and would probably have performed better if planted when smaller.

Capparis tomentosa, Albizia coriaria and *Warburgia salutaris* grew slowly in our experiment. The first two are dry land or dry forest species and may be slow growing when they establish naturally as well. Even if they do not qualify as FWS, it is important to include them in these tree gardens as they are important medicinal plants and are becoming rare. *W. salutaris* is a wet forest species, and probably suffered from the drier climate in Kaliro. A cultivation experiment in South Africa has proved that a key to success for planting medicinal plants is that the species are located in a similar site to their natural habitat (Mander et al. 1996).

There is a debate as to whether cultivation is the best way of conserving medicinal plants and securing supply. It has been argued that collection from the wild will continue to be the best option, simply because it costs less money and is time efficient (Schippmann et al. 2002). However, small-scale cultivation that requires low economic inputs could be a good way of responding to decreasing local stocks, generating income and supplying local and regional markets. Nevertheless, cultivated medicinal plants are regarded as less valuable in some cultures; it is believed that they 'lose power' if cultivated (Wiersum et al. 2006). Scientific studies partly support this belief. The medicinal effects of plants usually depend on the presence of secondary metabolites which the plants produce to protect themselves against enemies such as bacteria, fungi, insects and other herbivores. The quantity of compounds produced is related to the hazards of the natural environment. Large scale cultivation with the addition of fertiliser and water will reduce stress and thereby probably reduce the production of secondary metabolites (Schippmann et al. 2002).

The minimum acceptable performance standards proposed in this paper should not be regarded as absolute; they must be used with flexibility and the methods used adjusted to each area, as recommended by Elliott et al. (2003).

Conclusion

We found the Framework species method to be a useful tool to test which medicinal trees can be cultivated. In Uganda mainly *Eucalyptus* and *Pinus* species are planted both for timber production and restoration of forests. We believe that the FWS method is a good way of finding alternative candidates for community forests and restoration in a way that can satisfy different needs of the population and minimise the threat of habitat degradation and species extinction.

We propose planting a mixture of local medicinal tree species in a multipurpose tree garden, since this will probably not influence the chemical content of the planted species as much as planting in monocultures. However this will have to be tested in further work. At the same time, the tree garden would provide people with a wide variety of products needed such as food, fodder, fire-wood and building material in addition to medicinal plants.

In our research we tried to target problems experienced by traditional healers and the rural population. The healers received training, information about results, the nursery was handed over to them and they were left with around 1000 trees to manage and harvest. We believe that establishing community forests or private multipurpose tree gardens are good ways of securing the rural poor with much needed forest products, and counteract the deforestation; efforts needed in most African counties. We found the framework species method to be a useful tool to establish multipurpose community forests. However, support from governments or NGOs to provide knowledge and funding to establish small tree nurseries are needed.

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References

Adjanohoun JE, Ahyi MRA, Aké Assi L, Amai CA, Gbile ZO, Johnson CLA, Kakooko ZO, Lutakome HK, Morakinyo O, Mubiru NK, Ogwal-Okeng JW, Sofowora EA (1993) Contribution to Ethnobotanical and Floristic studies in Uganda. Scientific, Technocal and Research commission of the Organisation of African Unity (OAU/STRC)

Blakesley D, Elliot S, Kuarak C, Navakitbumrung P, Zangkum S, Anusarnsunthorn V (2002) Propagating framework tree species to restore seasonally dry tropical forest: implications if seasonal seed dispersal and dormancy. For Ecol Manage 164:31-38

Chhabra SC, Mahunnah RLA, Msiu EN (1987) Plants used in traditional medicine in Eastern Tanzania. I. Pteridophytes and Angiosperms (Acanthaceae to Canellaceae). J Ethnopharmacol 21:253-277

Cole D (2003) The impact of certification on the sustainable use of devil's claw (*Harpagophytum procumbens*) in Namibia. Paper prepared for FAO, Non-wood forest programme, p 34

Elliott S, Navakitbumrung P, Kuarak C, Zankum S, Anusarnsunthorn V, Blakesley D (2003) Selecting framework tree species for restoring seasonally dry tropical forests in northern Thailand based on field performance. For Ecol Manage 184:177-191

Elliot S, Blakesley D, Chairuangsri S (2008) Research for restoring Tropical Forest Ecosystems: A Practical Guide. www.forru.org. Accessed 2 February 2009

Geissler W, Harris SA, Prince RJ, Olsen A, Odhiambo RA, Oketch-Rabah H, Madiega PA, Andersen A, Molgaard P (2002) Medicinal plants used by mothers and children in Bondo district, Kenya. J Ethnopharmacol 83:39-54

Hamilton AC (2004) Medicinal plants, conservation and livelihoods. Biodiv Conserv 13:1477-1517.

Hawkins B (2008) Plants for life: Medicinal plant conservation and botanic gardens. Botanic Gardens Conservation International, Richmond, U.K.

Heine B, Heine I (1988) Plant concepts and plant use. An ethnobotanical survey of the Semi-Arid and Arid Lands of East Africa. Part I. Plants of the Chamus (Kenya). Kölner Beiträge

zur Entwicklungsländerforchung, vol. 6. Breitenbach Publishers, Saarbrücken & Fort Lauderdale.

Katende AB, Birnie A, Tengnäs B (1995) Useful trees and shrubs for Uganda. Regional Soil Conservation Unit (RSCU), Kenya.

Kisangau DP, Hosea KM, Joseph CC, Lyaruu HVM (2007) In vitro antimicrobial assay of plants used in traditional medicine in Bukoba rural district, Tanzania. Afr J Trad, Compl Altern Med 4:510-523

Kokwaro JO (1976) Medicinal plants of East Africa. East African Literature Bureau, Nairobi.

Krog M, Falcao MP, Olsen CS (2006) Medicinal plant markets and trade in Maputo, Mozambique. Forest & Landscape Working Papers No 16-2006, Danish Centre for Forest, Landscape and Planning, KVL, Denmark.

Lye KA, Bukenya-Ziraba R, Tabuti JRS, Waako PJ (2008) Plant.Medicinal Dictionary for East Africa. Makerere Herbarium Handbook no. 2. Makerere University, Kampala, Uganda

Mander M, Mander J, Breen C (1996) Promoting the cultivation of indigenous plants for markets. Experiences from KwaZulu-Natal, South Africa, in Leakey RRB, Temu AB, Melnyk M, Vantomme P (Eds.), Domestication and commercialization of non-timber forest products in agroforestry systems, Rome, FAO (Non-wood forest products 9), pp. 104-109

NEMA (National Environmental Management Authority) (2002) State of the Environment Report for Uganda 2002. National Environmental Management Authority, Kampala, Uganda.

Neuwinger HD (1996) African Ethnobotany. Poisons and Drugs. Chapman & Hall, Weinheim.

Okullo JBL, Hall JB, Obua J (2004) Leafing, flowering and fruiting of *Vitellaria paradoxa* subsp. *nilotica* in savanna parklands in Uganda. Agrofor Syst 60:77-91

Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues. Inter-Department Working Group on Biology Diversity for Food and Agriculture, FAO, Rome.

Ssegawa P, Kasenene JM (2007) Medicinal plant diversity and uses in the Sango Bay area, Southern Uganda. J Ethnopharmacol 113:521-540

Stangeland T, Tabuti JRS, Lye KA (2007). The influence of light and temperature on the germination of two Ugandan medicinal trees. Afr J Ecol 46:565-571

Tabuti JRS (2007) The uses, local perceptions and ecological status of 16 woody species of Gadumire Sub-county, Uganda. Biodiv Conserv 16:1901-1915

Tabuti JRS, Lye KA, Dhillion SS (2003) Traditional herbal drugs of Bulamogi, Uganda: Plants, use and administration. J Ethnopharmacol 88(1):19-44

Tabuti JRS, Ticktin T, Arinaitwe MZ, Muwanika VB (2009) Community attitudes and preferences towards woody species and their implications for conservation in Nawaikoke Sub-count, Uganda. Oryx 43(3):393-402

Ticktin T (2004) The ecological implications of harvesting non-timber forest products. J of Appl Ecol 41:11-21

Tucker NIJ, Murphy TM (1997) The effects of ecological rehabilitation on vegetation recruitment: some observation from the wet Tropics of North Queenland. For Ecol Manage 99:133-152

van Wyk B-E (2008) A broad review of commercially important southern African medicinal plants. J Ethnopharmacol 119:342-355

Walter S (2001) Non-Wood Forest Products. In: Africa: A regional and National Overview. FAO working paper. FOPW/01/1

Wiersum KF, Dold AP, Husselman M, Cocks M (2006) Cultivation of medicinal plants as a tool for biodiversity conservation in the Amatola Region, South Africa, in Bogers, R.J., Craker, L.E., Lange, D. (Eds.), Medicinal and Aromatic Plants. Springer, Netherlands, pp. 43-57

Table 1.

Phenology data recorded every second week for some indigenous medicinal trees in Uganda

Species	Flowering	Fruiting
Sarcocephalus latifolius	Not observed	Fruited in December 2006. Fruited again in February through to mid August 2007
Securidaca longipedunculata	18 of 26 trees flowered at the beginning of April. All flowers were destroyed by a hailstorm on the 1st of May 2007.	8 of 27 trees fruited in November - December 2006.
Psorospermum febrifugum	Flowered from April to July 2007	Fruited from April to November.
Warburgia salutaris	June- August 2006	Peak fruiting was recorded in September 2006 and March 2007.

Table 2. Seed germination data for potential framework tree species in East Uganda

Scientific name	Local name	Family	Major use[a]	Seed collection month	% germination	MLG	Synchrony of germinat.	Germination and synchrony categories[b]
Acacia macrothyrsa	Muwologoma	Mimosaceae	M	September	18	12	7	RG/S
Albizia coriaria	Musita	Mimosaceae	T, M, Ch	May	56	9	21	RG/S
Annona squamosa	Mustaferi	Annonaceae	F, M	November	43	33	24	IG/IS
Artocarpus heterophyllus	Fene	Moraceae	F	July	61	21	27	RG/IS
Calliandra calothyrsus	Calliandra	Mimosaceae	SI, Fod	Seeds: NTSC	57	12	21	RG/S
*Callistemon citrinus**	Bottle brush	Myrtaceae	Fw, M	April	80	15	38	RG/IS
Calpurnia aurea	Lumanyo	Rabaceae	M. Fw	September	19	15	27	RG/IS
Canarium schweinfurthii	Mpafu	Burseraceae	T, F, M	October	100	50	39	IG/IS
Capparis tomentosa	Muzingani	Capparidaceae	M	August	100	10	14	RG/S
Carica papaya	Papali	Caricaceae	F, M	July	88	17	12	RG/S
Carissa spinarum	Mutyoga	Apocynaceae	F, M	August	68	38	6	IG/S
Ceiba pentandra	Mufamba	Bombacaceae	M, Fod	June	8	45	21	IG/S
Entada abyssinica	Musambamadhi	Mimosaceae	M	April	46	27	1	IG/S
Erythrina abyssinica	Musitisiti	Fabaceae	M	April	60	23	21	RG/IS
Eugenia jambos	Muzabibu	Myrtaceae	F	April	60	na	na	na
Ficus natalensis	Mukosi	Moraceae	M, S, bark cloth	cuttings	na	na	na	na
Ficus sycomorus	Mukunyu	Moraceae	S, M	July seeds/cutting	7	20	1	RG/S
Leucaena leucocephala	Lusina	Mimosaceae	Si	March	83	31	18	IG/S
Maesopsis eminii	Musizi	Rhamnaceae	T, M	March	100	31	18	IG/IS
Markhamia lutea	Musambya	Bignoniaceae	T, Th, M	March	65	11	10	RG/S
Milicia excelsa	Muvule	Moraceae	T, Hc	March	60	24	14	IG/S
*Sarcocephalus latifolius****	Mutamatama	Rubiaceae	M	December	60	15	15	RG/S
Senna siamea	Gassia seed	Caesalpiniaceae	M, Fw	May	39	47	16	IG/IS
Senna grandis	No local name	Caesalpiniaceae	Fw	May	16	29	93	IG/AS
Senna spectabilis	Gassia kibiliti	Caesalpiniaceae	Fw, Ch, Th	April	39	8	30	RG/IS
Terminalia schimperiana	Mukonge	Combretaceae	M	April	32	na	na	na
*Warburgia salutaris** **	Balwegira	Canellaceae	M, T	March	32	33	67	IG/IS

[a] Major use: medicine, M; charcoal, Ch; food, F; timber, T; firewood, Fw; soil improvement, Si; shade, S; fodder, Fod; tool handels, Th; house construction, Hc;
[b] RG: rapid germination; IG: intermediate germination; SG: slow germination; S: synchronous ; IS: intermediate synchrony; AS: asynchronous.
* Sowing experiment at National tree seed centre; ** Tree growing in tropical wet forest; *** laboratory experiment (Stangeland et al. 2008)

Table 3

Survival, mean tree height and mean crown width 13 months after planting of tree species

Scientific name	Nr of trees planted	Initial number monitored	% survival	n [a]	Mean height(cm)[b]	Mean crown width (cm)[b]
Acacia macrothyrsa (TS 214)	13	13	23,1	3	98 (36)	109 (36)
Albizia coriaria (TS 119)	60	28	75	21	64 (5)	57 (4)
Annona squamosa (TS 365)	39	23	17,4	4	74 (8)	35 (6)
Artocarpus heterophyllus (JRST 459)	60	30	80	24	127 (9)	59 (8)
Calliandra calothyrsus (TS 352)	15	14	92,9	13	257 (14)	192 (26)
*Callistemon citrinus (*TS 344)	45	30	73,3	22	185 (18)	112 (11)
Calpurnia aurea (TS 219)	45	27	25,9	7	125 (18)	78 (14)
Canarium schweinfurthii (JRST 538)	36	24	12,5	3	59 (11)	54 (14)
Capparis tomentosa (TS 5,9,10,118)	57	27	74,1	20	47 (4)	42 (5)
Carica papaya (JRST 506)	20	16	75	12	204 (14)	203 (16)
Carissa spinarum (TS 348)	60	30	93,3	28	147 (8)	160 (7)
Ceiba pentandra (TS 202)	60	28	100	28	235 (9)	149 (8)
Entada abyssinica (TS 349)	45	29	62,1	18	129 (18)	76 (12)
Erythrina abyssinica (JRST 26)	30	29	72,4	21	75 (8)	53 (8)
Eugenia jambos (TS 204)	60	28	78,6	22	71 (4)	53 (5)
Ficus natalensis (JRST 477)	21	19	42,1	8	77 (11)	29 (7)
Ficus sycomorus (JRST 472)	33	28	92,9	26	168 (10)	127 (9)
Leucaena leucocephala (TS 360)	60	29	93,1	27	222 (12)	157 (13)
Maesopsis eminii (TS 355)	30	20	10	2	132 (52)	134 (67)
Markhamia lutea (TS 398)	60	30	93,3	28	144 (11)	82 (7)
Milicia excelsa (JRST 500)	60	30	86,7	26	108 (4)	75 (5)
Sarcocephalus latifolius (TS1,3,16)	90	29	96,7	29	211 (15)	264 (18)
Senna siamea (JRST 262)	45	28	92,9	26	319 (18)	257 (24)
Senna sp.(TS 362)	30	30	60	18	120 (16)	115 (19)
Senna spectabilis (TS 343)	12	12	83,3	10	409 (48)	230 (41)
Terminalia schimperiana(TS 351, 354)	18	18	100	18	155 (7)	147 (8)
Warburgia salutaris (TS 109)	60	26	42,3	11	84 (9)	63 (7)

[a] Subsamples of surviving trees

[b] Values in brackets represent SE of mean

Table 4.

Summary of **Framework species classification** based on field performance (E, excellent; G, good; L, low; A, acceptable; M, marginally acceptable; U, unacceptable and R, rejected)

Species	% germination[a]	Survival[b]	Growth[c]	Crown width[d]	Overall classification
Acacia macrothyrsa	L	U	M	M	M
Albizia coriaria	M	E	U	M	A
Annona squamosa	M	U	M	U	R
Artocarpus heterophyllus	G	E	A	M	E
Calliandra calothyrsus	M	E	E	E	E
Callistemon citrinus	G	E	E	A	E
Calpurnia aurea	L	U	A	M	M
Canarium schweinfurthii	G	U	U	M	M
Capparis tomentosa	G	E	U	U	M
Carica papaya	G	E	E	E	E
Carissa spinarum	G	E	A	E	E
Ceiba pentandra	L	E	E	E	A
Entada abyssinica	M	A	A	M	A
Erythrina abyssinica	G	E	U	M	A
Eugenia jambos	G	E	U	M	A
Ficus natalensis		M	M	U	M
Ficus sycomorus	L	E	E	A	A
Leucaena leucocephala	G	E	E	E	E
Maesopsis eminii	G	U	A	A	A
Markhamia lutea	G	E	A	M	E
Milicia excelsa	G	E	M	M	A
Sarcocephalus latifolius	G	E	E	E	E
Senna siamea	M	E	E	E	E
Senna sp.	L	A	A	A	M
Senna spectabilis	M	E	E	E	E
Terminalia schimperiana	M	E	A	E	E
Warburgia salutaris	M	M	U	M	M

[a] G > 60%, M = 20-59.9%, L < 20%
[b] E >70%, A = 50 - 69.9%, M=40-49.9%, U <40%
[c] E > 1.6 m, A = 1.0 - 1.59, M = 0.6 - 0.99, U < 0.59
[d] E > 1.4 m, A = 1.0 - 1.39 m, M= 0.6 - 0.99 m, U < 0.6 m

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Total antioxidant activity in 35 Ugandan fruits and vegetables

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ABSTRACT

The objective of this study was to analyse antioxidant activity (AA) in fruits and vegetables from Uganda and to investigate whether AA in traditional food is sufficiently high to prevent oxidative stress and thus combat disease. We used the FRAP (ferric reducing ability of plasma) procedure. The results showed great variation in AA, ranging from 72.3 ± 13.5 (*Syzygium cuminii* seed) to 0.09 ± 0.05 (*Cucurbita maxima* fruit) mmol/100 g fresh weight (FW). We estimated serving sizes and determined the total antioxidant capacity (TDAC) per day of three traditional Ugandan diets. The dietary plants with highest AA per serving size were pomegranate (*Punica granatum*), *Canarium schweinfurthii*, guava (*Psidium guajava*), mango (*Mangifera indica*) and tree tomato (*Cyphomandra betacea*) with values ranging from 8.91 to 3.00 mmol/serving. Of the traditional diets, the central/eastern (C/E) and the western (W) diets had almost the same AA (9.31–9.78 and 9.75 mmol/day), while the northern (N) diet had an AA of 7.50–8.02 mmol/day.

1. Introduction

A diet rich in fruits, vegetables and minimally refined cereals is associated with lower incidence of illnesses such as coronary heart disease, some forms of cancer and neurodegenerative ailments (Dragsted et al., 2006; WHO/FAO, 2003). Plants produce a wide range of redox-active secondary metabolites (i.e. antioxidants) such as ascorbic acid, carotenoids, polyphenols and enzymes with antioxidant activity, which protect the cells from oxidative damage. In animal cells, the production of antioxidants is much more limited (Halvorsen et al., 2006). Free radicals are constantly generated in the body as a result of oxidative metabolism. Certain diseases, smoking, environmental poisons, alcohol and ionising radiation promote the generation of free radicals. Oxidative stress occurs if the antioxidant defence in the organism is not adequate (Blomhoff, Carlsen, Andersen, & Jacobs, 2006). Various studies have demonstrated a close link between oxidative stress and development of different ailments, such as inflammatory conditions, cancer, AIDS, gastric ulcer, hypertension and neurodegenerative ailments (Halvorsen et al., 2002; Hegde, Rajasekaran, & Chandra, 2005; Papaharalambus & Griendling, 2007).

The human body has an antioxidant defence system, and it has been assumed that a diet rich in antioxidants strengthens this system. It has been postulated that a network of antioxidants with different chemical properties may work in a synergistic way, protecting the cells from damage (Blomhoff, Carlsen, Andersen, & Jacobs, 2006). Fruits, including berries and nuts, some seeds, vegetables, and some beverages (coffee, tea, red wine and fruit juices) are good sources of antioxidants.

Bioavailability and absorption of different kinds of antioxidants in the human body are still poorly understood. While some antioxidants are easily absorbed and their manner of action is fairly well known (e.g. vitamin C), recent research indicates that polyphenols do not work in vivo as antioxidants in the conventional way (Stevenson & Hurst, 2007) but instead, provide substantial protection against oxidative stress by inducing cellular endogenous enzymic protective mechanisms. They appear to be able to regulate antioxidant enzyme gene transcription and numerous aspects of intercellular signalling cascades involved in the regulation of cell growth, inflammation and many other processes. β-Carotene (provitamin A carotenoids) is available in green leafy and yellow–orange vegetables and fruits. In a study of six cultivars of Indian mangoes and two cultivars of papaya, it was found that the content and bioaccessibility of β-carotene varied among cultivars and that mango gave amounts of β-carotene three times higher than those found in papaya (Veda, Platel, & Srinivasan, 2007). In India, these two fruits are frequently used in milkshakes, which were shown to increase bioaccessibility of β-carotene. Vitamin A insufficiency is one of the main micronutrient deficiencies in developing countries (WHO/FAO; 2003). Since the various groups of antioxidants function differently, the values of total AA are not directly comparable.

Processing has an effect on AA of some dietary plants. For some vegetables, such as carrots, spinach, mushrooms, peppers, potatoes, sweet potatoes, cabbage, broccoli and tomatoes, AA increased when these vegetables were boiled or steamed (Halvorsen et al.,

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2006; Potsędek, 2007). Aoyama and Yamamoto (2007) found that AA in green Welch onion (*Allium fistulosum*) increased during boiling, while it decreased in two varieties of onion (*Allium cepa*, yellow and red) and white-sheath Welch onion. The major flavonoid in yellow and red onions is quercetin, and in green Welch onion it is kaempferol. This indicates that the effect of boiling on antioxidant activity in dietary plants depends on the compound that generates the antioxidant activity.

In the western world, enormous efforts have been directed in the past two decades toward research and information on the role of nutrition and diet on health while, in developing countries, this topic has received relatively little attention. Uganda is plagued by health problems, including high rates of malaria, HIV/AIDS, tuberculosis and malnutrition. About 40% of children below 5 years of age have stunted growth (WHO, 2007), and there is an extremely high prevalence of anaemia among children and adolescent women (73% of children and 49% of women between 15 and 49 years of age; UBOS, 2006), of which nearly half is due to iron deficiency (Totin et al., 2002). Chronic diseases, such as cancer, cardiovascular diseases and diabetes, are also spreading and are expected to increase in the coming decennia (WHO/FAO, 2003).

In this study we have investigated the antioxidant activity (AA) in some commonly consumed fruits, seeds and vegetables in Uganda. We have compared AA in the fruits from different geographical regions, paying special attention to fruits and vegetables not previously analysed. We have also calculated the AA per serving size (mmol/serving) and used this to estimate the daily intake of antioxidants in three traditional Ugandan diets.

2. Materials and methods

2.1. Plant material

The fruits and vegetables were either harvested in registered localities or bought at local markets in Uganda from March 2005 to May 2007. After each collection, the samples were analysed within 6 months after harvest. Between two and 15 samples of each species were collected. Within a few hours after collection, the samples were frozen at −20 °C and brought frozen to Norway for antioxidant analysis (FRAP assay).

2.2. Extraction of the plant material

The edible parts of the fruits/vegetables were analysed. In addition, seeds of Java plum (*Syzygium cuminii*), which are used as medicine, were analysed. The procedure was performed as described in Remberg, Haffner, and Blomhoff (2003). Slightly thawed samples (approximately 30 g) were mashed with a hand-held blender. Triplicate samples of 3 g of each sample were diluted in 30 ml of methanol and flushed with nitrogen in order to prevent oxidation. The bottles were exposed to sonication at 0 °C for 15 min in an ultrasonic bath and then stored frozen (−20 °C) until analysed.

2.3. Automated FRAP assay

Antioxidant activity in the samples was measured using the ferric reducing ability of plasma (FRAP) assay (Benzie & Strain, 1996), following the modification by Halvorsen et al. (2002) in which the samples were diluted in methanol instead of water. The FRAP method measures the absorption change that appears when the TPTZ (2,4,6-tri-pyridyl-*s*-triazine)-Fe^{3+} complex is reduced to the TPTZ-Fe^{2+} form in the presence of antioxidants. An intense blue colour, with absorption maximum at 593 nm, develops. Our measurements were performed at 595 nm with an incubation time of 10 min at 37 °C. The FRAP method gives a measure of the total antioxidant activity of the sample. A triplicate of each sample was analysed using the KoneLab 30i (Kone Instruments Corp., Espoo, Finland) spectrophotometer. The instrument was calibrated on a daily basis using Fe $SO_4 \cdot 7H2O$ (10 mM), with trolox (500 μM) as a control. The antioxidant activity in the samples was calculated as mmol Fe^{2+} per 100 g fresh weight (mmol/100 g FW) of sample.

2.4. Estimated serving sizes

The National Nutrient Database for Standard Reference contains information on some dietary plants and their serving sizes as established by the US Food and Drug Administration (USDA, 2002). For plants not registered in the database, serving sizes were determined on the basis of actual measurements of average portion weights taken during sample preparation. Assistance was provided by informants native to the three Ugandan regions studied. The antioxidant content per serving size was then calculated.

2.5. Data analysis

Oneway analysis of variance (ANOVA) was used to test for differences in antioxidant activity between the different dietary plants.

3. Results and discussion

3.1. General

The total antioxidant activity in the methanolic extracts from 35 Ugandan fruits and vegetables was assessed. The results show great variation within the different fruits and vegetables, ranging from 0.04 to 93.7 mmol 100 g^{-1} FW (Table 1a and b).

3.2. Fruits and seeds

The seed of Java plum (*Syzygium cuminii*) had the highest antioxidant activity (72.3 ± 13.5 mmol 100 g^{-1}) of all the species analysed (Table 1a). Although Java plum is not a species native to Uganda, it is commonly planted in gardens. In central Uganda, the dried and powdered seeds of Java plum have traditionally been used as herbal medicine to treat asthma (Wanyana-Maganyi, pers. comm.). In a survey of 133 Indian medicinal plants, the seed of Java plum was ranked as number nine in antioxidant activity, measured by the FRAP assay (Surveswaran, Cai, Corke, & Sun, 2007). This seed may have other potential uses for improving health, and should be further investigated for active ingredients, efficacy, and safety as medicine (WHO, 2003). In addition to the seed, the fruit pulp was also found to be rich in antioxidants (3.32 ± 3.66 mmol 100 g^{-1}; Table 1a). The Java plum is a popular but perishable fruit, and is only available at local markets during short periods. The large variation in FRAP values of the pulp could be due to different cultivars, or growing or storage conditions.

African elemi (*Canarium schweinfurthii*) is a large tropical tree, widely distributed from Sierra Leone in West Africa to the eastern region of the continent and south to Angola. The fruits ripen in the dry season and become hard and uncookable only 4–6 days after harvesting. The pulp contains 36–58% of vegetable oil (Thiégang, Leudeu, Kapseu, Fomethe, & Parmentier, 2004) and 28.8% of the pulp oil is polyunsaturated fatty acids. Hard fruits can still be used for oil production and, in West Africa, the fruits have been studied as a potential basis for small-scale production of cooking oil (Abayeh, Abdulrazaq, & Olaogun, 1999; Thiégang et al., 2004), and could also potentially be used for cooking oil production in Uganda. The seed of this fruit is also consumed, and AA of the seed is relatively high (4.77 ± 2.58 mmol 100 g^{-1}). Among the fruits,

Table 1
Total antioxidant activity in Ugandan plants

Botanical name	Fruits	Part/state of plant	Family	*n*	Antioxidant activity	Range
(a) Total antioxidant activity in Ugandan fruits and seeds						
Syzygium cuminii (L.) Skeels	Java plum	Raw Seed	Myrtaceae	7	72.3 ± 13.5	56.0–93.7
Canarium schweinfurthii Engl.	African elemi	Raw fruit	Burseraceae	10	45.3 ± 17.6	19.1–74.6
Vangueria apiculata L.	Vangueria	Unripe fruit	Rubiaceae	4	27.1 ± 8.43	19.1–39.0
Vangueria apiculata L.	Vangueria	Semi-ripe fruit	Rubiaceae	2	12.3 ± 9.30	5.73–18.9
Punica granatum L.	Pomegranate	Ripe fruit	Punicaceae	3	10.5 ± 3.44	7.04–13.9
Canarium schweinfurthii Engl.	African elemi	Boiled fruit	Myrtaceae	9	5.31 ± 3.36	1.61–11.9
Punica granatum L.	Pomegranate	Semiripe fruit	Punicaceae	4	5.11 ± 0.61	4.31–5.78
Canarium schweinfurthii Engl.	African elemi	Raw Seed	Myrtaceae	6	4.77 ± 2.58	1.26–7.54
Syzygium cuminii (L.) Skeels	Java plum	Ripe fruit	Myrtaceae	8	3.32 ± 3.66	1.42–12.2
Psidium guajava L.	Guava	Ripe fruit	Myrtaceae	8	3.21 ± 0.80	2.90–4.44
Capsicum fruticosum L.	Chilli pepper	Ripe fruit	Solanaceae	9	2.91 ± 0.72	1.73–3.97
Canarium schweinfurthii Engl.	African elemi	Boiled seed	Burseraceae	3	2.96 ± 0.68	2.19–3.45
Tamarindus indica L.	Tamarind	Ripe fruit	Fabaceae	9	1.88 ± 1.18	0.91–4.55
Mangifera indica L.	Mango	Ripe fruit	Anarcadiaceae	11	1.62 ± 0.78	0.75–2.77
Cyphomandra betacea (Cav.) Sendth.	Tree tomato	Ripe fruit	Solanaceae	9	1.62 ± 0.27	1.31–1.96
Musa x paradisiaca L.	Banana (small)	Ripe fruit	Musaceae	13	1.40 ± 2.64	0.17–8.66
Citrus x aurantium L.	Orange	Ripe fruit	Rutaceae	11	1.28 ± 0.34	0.77–1.95
Carica papaya L.	Papaya	Ripe fruit	Caricacaea	11	0.89 ± 0.45	0.43–1.82
Passiflora edulis Sims	Passion fruit	Ripe fruit	Passifloriaceae	11	0.72 ± 0.19	0.52–1.05
Phaseolus vulgaris L.	Brown beans	Ripe fruit	Fabaceae	2	0.71 ± 0.04	0.68–0.74
Citrus x limon (L.) Burm.f.	Lemon	Ripe fruit	Rutaceae	9	0.69 ± 0.28	0.49–1.43
Solanum anguivi Lam.	Small bitter tomato	Ripe fruit	Solanaceae	8	0.61 ± 0.22	0.33–0.92
Musa x paradisiaca L.	Banana (big)	Ripe fruit	Musaceae	9	0.58 ± 0.51	0.26–1.91
Arachis hypogaea L.	Groundnut	Ripe fruit	Fabaceae	3	0.51 ± 0.15	0.39–0.68
Physalis peruviana L.	Physalis	Ripe fruit	Solanaceae	12	0.39 ± 0.12	0.21–0.58
Capsicum annuum L.	Sweet pepper	Ripe fruit	Solanaceae	4	0.38 ± 0.10	0.32–0.52
Solanum lycopersicum L.	Tomato	Ripe fruit	Solanaceae	10	0.37 ± 0.12	0.15–0.51
Vangueria apiculata L.	Vangueria	Ripe fruit	Rubiaceae	6	0.35 ± 0.18	0.19–0.59
Persea americana Mill.	Avocado	Ripe fruit	Lauraceae	9	0.34 ± 0.10	0.22–0.51
Annanas sativum Merr.	Pinapple	Ripe fruit	Bromeliaceae	10	0.33 ± 0.08	0.22–0.50
Solanum aethiopicum L.	Bitter tomato	Ripe fruit	Solanaceae	9	0.31 ± 0.20	0.08–0.63
Musa x sapientum L.	Matoke	Ripe fruit	Musaceae	9	0.22 ± 0.10	0.12–0.46
Phaseolus vulgaris L.	French beans	Unripe fruit	Fabaceae	12	0.21 ± 0.10	0.14–0.48
Ipomoea batatas (L.) Lam.	Sweet potato	Tuber	Convolvulaceae	2	0.15 ± 0.04	0.12–0,18
Artocarpus heterophyllus Lam.	Jackfruit	Ripe fruit	Moraceae	8	0.15 ± 0.07	0.07–0.28
Solanum melongena L.	Eggplant	Ripe fruit	Solanaceae	11	0.15 ± 0.10	0.05–0.40
Cucurbita maxima Lam.	Pumpkin	Ripe fruit	Cucurbitaceae	11	0.09 ± 0.05	0.04–0.17
(b) Antioxidant activity in green leafy vegetables						
Cleome gynandra L.	Spiderplant	Leaves	Capparidaceae	10	1.56 ± 0.73	0.53–2.92
Amaranthus spp.	Amaranth	Leaves	Amaranthaceae	15	1.00 ± 0.32	0.27–1.51
Solanum macrocarpon L.	Solanum	Leaves	Solanaceae	9	0.87 ± 0.17	0.63–1.16
Spinacia oleracea L.	Spinach	Leaves	Chenopodiaceae	9	0.98*	

* Halvorsen et al., (2002).

Canarium schweinfurthii had the highest AA (45.3 ± 17.6 mmol 100 g^{-1}). However, this fruit is only consumed after being soaked in hot water for 30 min. We found that during soaking, AA declined from 45.3 to 5.31 ± 3.36 mmol 100 g^{-1}. Consequently, new methods need to be developed in order to cold press oil from the fruits. This may be a good way of using the excess fruits in the fruiting season while providing good quality cooking oil at the same time.

Pomegranate (*Punica granatum*) is regarded as a very good source of antioxidants (Lansky & Newman, 2007). In this study, AA was 10.5 ± 3.44 mmol 100 g^{-1} for ripe fruits, which is similar to results found by Halvorsen et al. (2002). The fruit is not common in Uganda, and it is usually not fully ripe when sold in markets. In this study, the semiripe pomegranates had lower antioxidant activity than had the ripe ones (Table 1a), which corresponds with findings made by Kulnarni & Aradhya (2005). In brown beans we also found AA to be higher in the ripe seeds than in unripe fruits (Table 1a). Navarro, Flores, Garrido, and Martinez (2006) found similar results for sweet pepper fruits (*Capsicum annuum*); their highest antioxidant activity was at the mature, red state. In contrast, AA for *Vangueria apiculata* fruits decreased considerably as the fruit ripened (Table 1a). This indicates that AA increases in some fruits during maturation, but decreases in others. This could be due to different kinds of antioxidant compounds causing high AA, e.g. tannins, which are often more abundant in unripe fruits, while the opposite is often the case for anthocyanins.

Fruits of the pea family (Fabaceae) had medium to high amounts of AA (groundnuts 0.51 ± 0.15, and brown beans 0.71 ± 0.04 mmol 100 g^{-1} FW), but they could be important antioxidant sources as they are common in the Ugandan diet and are consumed frequently.

3.3. Wild leafy vegetables

The wild leafy vegetables were all good sources of antioxidants. Spiderplant (*Cleome gynandra*), *Amaranthus* spp. and *Solanum macrocarpon* had antioxidant activities of 1.56, 1.00 and 0.87 mmol 100 g^{-1} FW, respectively (Table 1b). The wild greens are frequently harvested in rural areas. They are also grown in home gardens, as cash crops, and are available at markets in the cities.

3.4. Comparing results

For most of the species we studied, our results correspond with those of Halvorsen et al. (2002), but they differ considerably for mango and pineapple from Costa Rica (Table 2). On average, the mangoes from our study have an antioxidant activity more than

Table 2
Antioxidant activity in selected fruits found in present and reference study

Common name	Antioxidant content		Place of origin in reference study
	Present study(mmol/100 g)	Reference study*(mmol/100 g)	
Pomegranate	10.5	11.3	Spain
Chilli pepper	2.91	2.46	Spain, Holland
Mango	1.62	0.35	S. America, Pakistan
Orange	1.28	1.14	Spain
Lemon	0.69	1.02	Spain
Banana	0.58	0.2	Costa Rica
Tomato	0.37	0.31	Netherlands, Spain, Italy, Mali
Avocado	0.34	0.41	Spain, Israel
Pineapple	0.33	0.39	Ivory Coast
		1.36	Costa Rica
Eggplant	0.15	0.17	Netherlands, Italy, Mali

* Halvorsen et al. (2002).

four times higher than the mangoes from South America in the Halvorsen study. On the other hand, pineapples from Costa Rica, analysed by Halvorsen et al. (2002), had AA values more than three times higher than those observed in Uganda (Table 2). However, our results are in agreement with Halvorsen et al. (2002) for pineapples from the Ivory Coast in West Africa, which had AA values similar to those in our study. These differences may be attributed to different cultivars, growing conditions, stages of ripening at harvest, or the storage conditions and time elapsed before the fruits were analysed.

3.5. The antioxidant content per serving size

Serving sizes of food in Uganda vary considerably, depending on season, availability, food traditions in different parts of the country, people's knowledge, capacity and economy.

The edible state of the plants is included in Table 3 (antioxidant content per serving size), e.g. ripe fruits of pomegranate and *Vangueria* and boiled *Canarium* fruit. The top five fruits in decreasing order are pomegranate, boiled *Canarium* fruit, guava, mango and tree tomato, with antioxidant activities of 8.91, 5.52, 5.30, 3.35 and 3.00 mmol/serving size, respectively. Maize, sweet potato and groundnuts had AA values of 0.42, 0.39 and 0.31 mmol/serving size, respectively, but, as these plants are frequently grown and are fairly inexpensive in season, they are eaten in quantities larger than the normal serving size, and therefore are relatively more important as antioxidant sources.

3.6. Contribution of different food groups to intake of antioxidants in three Ugandan diets

We have estimated the total dietary antioxidant capacity (TDAC) in three traditional diets in Uganda, namely from the central/eastern (C/E), northern (N) and western (W) parts of the country. The values are relevant for situations in which there is no drought or famine and the family has been able to grow enough food or have money enough to buy food. Kiguli-Malwadde and Kasozi (2002) found that people in Uganda tend to maintain their dietary traditions from their homesteads even if they move to urban areas. On the other hand, Raschke and Cheema (2008) found

Table 3
Antioxidant activity of Ugandan food per serving size

Species	N	Antioxidant activity (mmol/100 g)	SD g	Serving size	Serving description	Antioxidant activity (mmol/serving)
Pomegranate	3	10.5	3.44	85	1 fruit	8.91
Canarium fruit boiled	9	5.31	3.36	104	2 handful	5.52
Guava	8	3.21	0.8	165	3 medium fruits	5.3
Mango	11	1.62	0.78	207	1 fruit	3.35
Tree tomato	9	1.62	0.27	185	5 fruits	3
Syzygium fruit	8	3.32	3.66	49	1 handful	1.63
Orange	11	1.28	0.34	110	1 fruit	1.41
Papaya	11	0.89	0.45	150	1 cup sliced	1.34
Spiderplant (*Cleome*)	10	1.56	0.73	76	1 cup	1.19
Banana (small)	13	1.4	2.64	70	2 fruits	0.98
Matoke banana	9	0.22	0.1	395	5 fruits	0.87
Brown beans	2	0.71	0.04	120	8 pers.: 1 kg	0.85
Solanum macrocarpon	9	0.87	0.17	75	1 cup	0.65
Amaranth	15	1	0.32	65	1 cup	0.65
Lemon	9	0.69	0.28	80	1 fruit	0.55
Pineapple	10	0.33	0.08	155	1 cup sliced	0.51
Tomato	10	0.37	0.12	123	1 fruit	0.46
Banana (big)	9	0.58	0.51	75	1 fruit	0.44
Maize	2	0.22	0.05	190	1 maizecob	0.42
Passion fruit	11	0.72	0.19	56	2 fruits	0.4
Sweet potato	2	0.15	0.04	260	2 potatoes	0.39
Physalis	12	0.39	0.12	85	1/2 cup	0.33
Groundnut	3	0.51	0.15	60	8 pers.: 1/2 kg	0.31
Avocado	9	0.34	0.1	90	1/2 fruit	0.31
Solanum aethiopicum	9	0.31	0.2	96	4 fruits	0.3
Jackfruit	8	0.15	0.07	165	1 cup	0.25
Canarium seed boiled	3	2.96	0.68	7	1 tsp.	0.21
Tamarind	9	1.88	1.18	8	2 medium fruits	0.15
Solanum anguivi	8	0.61	0.22	19	2 tblsp	0.12
Eggplant	11	0.15	0.1	70	1/2 fruit	0.11
Pumpkin	11	0.09	0.05	116	1/8 fruit	0.1
French beans	12	0.21	0.1	46	2 tblsp	0.1
Vangueria ripe	6	0.35	0.18	25	5 fruits	0.09
Chilli pepper	9	2.91	0.72	2	1 small fruit	0.06
Sweet pepper	4	0.38	0.1	10	1 tblsp	0.04

that the diets in East African countries, including Uganda, have undergone a major transition in recent decades, shifting from traditional local whole foods to easy-to-prepare foods bought at supermarkets. The globalised food system is gradually changing the traditional food habits in East Africa, which have many health benefits, and is fostering increased consumption of refined flour and sugar, inexpensive vegetable fats and food additives, which are known to hasten the development of non-communicable diseases (NCD) such as cancer, diabetes and cardiovascular diseases.

The estimates of TDAC in the three Ugandan diets show that the C/E and W diets have almost the same TDAC; 9.31–9.78 and 9.75 mmol, respectively (Tables 4a and b). In the C/E diet, vegetables are the single highest contributor to TDAC, with 5.52 mmol or 56%, followed by legumes (1.31 mmol), tea (1.25 mmol), and fruits (0.91 mmol) (Fig. 1). In the W diet, vegetables are still the

Table 4a
Antioxidant activity in average daily diet, central and eastern Uganda (Buganda & Busoga)

Meal	Species	Antioxidant activity (mmol/100 g)	Serving size (g)	Description of serving	Antioxidant activity (mmol/serving)	
					Low alternative	High alternative
Breakfast	Sweet potato or	0.33	130	1 medium		0.43
	Groundnut	0.51	30	1/2 cup	0.15	
Beverage	Tea	0.25	500	2 cups	1.25	1.25
	Milk**	0.14	250	1 cup	0.35	0.35
Lunch	Matoke	0.22	400	5 fruits	0.88	0.88
	Cassava* or	0.17	50	1/4 cup		0.09
	Rice*	0.17	44	1/4 cup	0.07	
	Fish** or	0.14	85		0.12	
	Groundnut sauce	0.51	60			0.31
	Amaranth	1	65	1 cup raw	0.65	0.65
	Solanum anguivi	0.61	19		0.12	0.12
	Curry powder**	9.98	2	1 tsp	0.2	0.2
	Tomato	0.37	125	1 fruit	0.46	0.46
	Onion*	0.67	50	1 small	0.34	0.34
	Passion fruit juice	0.72	30	1 fruit/ glass	0.22	0.22
Dinner	Matoke	0.22	400	5 fruits	0.88	0.88
	Cassava* or	0.17	60		0.1	
	Rice*	0.17	60			0.1
	Beans	0.71	120		0.85	0.85
	Amaranth	1	65		0.65	0.65
	Solanum anguivi	0.61	20		0.12	0.12
	Curry powder**	9.98	2		0.2	0.2
	Tomato	0.37	125	1 fruit	0.46	0.46
	Onion*	0.67	50	1 small	0.34	0.34
	Passion fruit juice	0.72	30	1 fruit/ glass	0.22	0.22
In between	Banana	0.58	75		0.44	0.44
	Jack fruit	0.15	165		0.25	0.25
	SUM				9.31	9.78

Vegetables: 5.52, legumes: 1.31, tea: 1.25, fruit: 0.91, spices: 0.4, milk: 0.35.

Table 4b
Antioxidant activity in average daily diet, northern Uganda (Acholi)

Meal	Species	Antioxidant activity (mmol/100 g)	Serving size (g)	Description of serving	Antioxidant activity (mmol/serving)	
					Low alternative	High alternative
Breakfast	Cassava	0.17	200	1 cup	0.34	0.34
	Sesame seeds, ground	1.21	15	1 tbsp	0.18	0.18
Beverage	Tea	0.25	500	2 cups	1.25	1.25
	Milk	0.14	250	1 cup	0.35	0.35
Lunch	Cassava*	0.17	200	1 cup	0.34	0.34
	Beans	0.71	120		0.85	0.85
	Sesame seeds	1.21	50		0.61	0.61
Dinner	Finger millet whole grain and	0.82	100	½ cup		0.82
	Cassava powder or	0.17	130	½ cup		0.22
	Sorghum and	0.3	100	½ cup	0.3	
	Cassava powder	0.17	130	½ cup	0.22	
	Beans	0.71	120		0.85	0.85
	Sesame seeds	1.21	50		0.61	0.61
	Greens	1	70	1 cup	0.7	0.7
	Passion fruit juice	0.72	30	1 fruit/ glass	0.22	0.22
In between	Banana	0.58	75	1 fruit	0.44	0.44
	Jack fruit	0.15	165	1 cup	0.25	0.25
	Sum				7.5	8.02

Legumes: 1.70, vegetables: 1.62, sesame seeds: 1.4, tea: 1.25, fruits: 0.89, grains: 0.82, milk: 0.35.

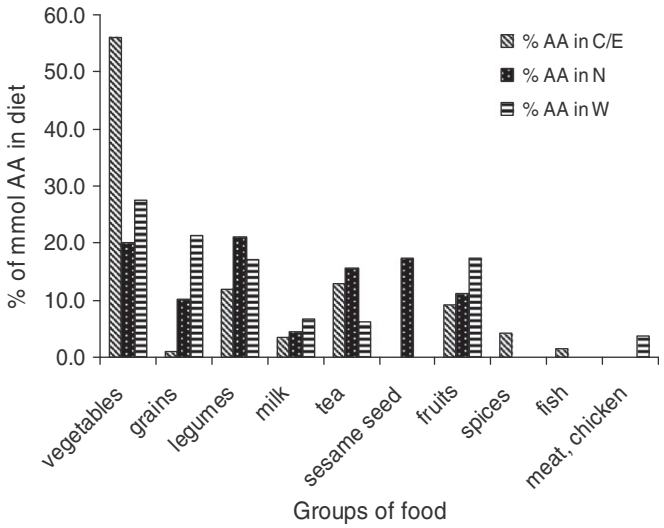

Fig. 1. Contribution of antioxidants in three traditional diets in Uganda, based on the following foods: vegetables, grains, legumes, milk, tea, sesame seeds, fruits, spices, fish and meat/chicken. C/E = central and eastern, N = northern, and W = western Uganda.

most important antioxidant source, contributing 2.47 mmol or 27.5%. But, in this part of the country, grains (finger millet) play a much more important role (2.13 mmol), followed by fruits (1.77 mmol), legumes (1.7 mmol), milk (0.67 mmol) and tea (0.63 mmol). The N diet has the lowest AA according to our calculations, giving 7.5–8.02 mmol per day (Table 4c, Fig. 1). However, this diet otherwise has a good profile, with varied amino acids and iron. The legumes are most important, giving 1.7 mmol or 21.2% of daily antioxidant activity, followed by vegetables (1.62 mmol), sesame seeds (1.4 mmol), tea (1.25 mmol), fruits (0.89 mmol) and grains (0.82 mmol). In case of drought or famine, the intake of food, and thus antioxidants, would be reduced to half or less. School children are also likely to consume far fewer antioxidants when they eat lunch at school, which mainly consists of maize porridge or cassava.

In a 7-day weighed dietary record in a group of 61 Norwegian adults, Svilaas et al. (2004) found that the average intake of antioxidants per day was 17.3 mmol. Surprisingly, coffee contributed 11.1 mmol or 64%, followed by fruits and berries, tea, wine, cereals and vegetables. If coffee is subtracted from the Norwegian diet, and tea from the Ugandan diets, we find that the Norwegians receive less AA (6.2 mmol) through food than that obtained through the Ugandan diets (6.8–9.12 mmol). The TDAC of Svilaas is in agreement with Pellegrini et al. (2007) for 285 people in northern Italy. Both in northern Italy and Spain, it was found that beverages, such as wine, coffee and tea, contribute significantly to AA in the diets (Saura-Calixto & Goñi, 2006). The relatively high intake of AA from food in Ugandan diets is probably due to a higher intake of plants, as compared with European diets. Plants are generally higher in AA than is food of animal origin. Even if AA from food in the traditional Ugandan diets is relatively high, TDAC with beverage included is only 50–60% of that obtained in the Norwegian diet. Uganda is among the poorest countries in the world with 35% of the population living under the poverty line (CIA, 2008), which means that they can barely afford 1 meal per day. Food prices are increasing, and land is becoming scarce as the population grows. Under these circumstances, it is important to make recommendations regarding what crops to grow and what foods to eat in an effort to fight malnutrition and improve health. Sub-Saharan Africa is under a double burden of disease, as the classical malnutrition-related and infectious diseases coexist with the NCD caused by western-influenced diet and lifestyle, especially in the urban areas. The food groups found to be most beneficial in preventing various chronic infectious diseases are fruits and vegetables (WHO/FAO, 2003).

There is still no recommendation for antioxidant consumption but, if we assume that the Norwegian intake is not too high, even a full Ugandan diet provides too little AA. If more portions of fruits or vegetables were added to the Ugandan diet, TDAC would come close to the intake of AA found in European diets (Table 3). In our study there is some uncertainty concerning values on finger millet and black tea. AA in black tea intake per day, reported by Svilaas et al. (2004), was 1.4 mmol, and his group used the value 2.5 mmol per cup of "ready to drink" tea. In Uganda, far fewer tea leaves per cup are used compared with Norway, i.e. about ¼, calculated to 0.63 mmol per cup of Ugandan tea. Several papers have established the importance of sorghum and finger millet as good sources of polyphenols and antioxidants (Rajasekaran, Nithya, Rose, & Chandra, 2004; Chetan & Malleshi, 2007). Some sorghum and finger millet varieties with prominent pigmented testa (brown, black, red) are rich in phenolic compounds and have

Table 4c
Antioxidant activity in average daily diet, western Uganda (Ankole)

Meal	Species	Antioxidant activity (mmol/100 g)	Serving size (g)	Description of serving	Antioxidant activity (mmol/serving) Low alternative
Breakfast /beverage	Milk	0.14	480	2 cups	0.67
	Finger millet porridge*	0.82	260	4 cups porridge	2.13
	Tea	0.25	250	1 cup	0.63
Lunch	Sweet potato boiled**	0.33	100	1	0.33
	Yam*	0.22	100		0.22
	Matoke	0.22	100	1 fruit	0.22
	Pumpkin	0.09	100	1/8 fruit	0.09
	Beans	0.71	120		0.85
	Cassava	0.17	50	1 piece	0.09
Dinner	Sweet potato boiled**	0.33	100	1 potato	0.33
	Yam*	0.22	100		0.22
	Matoke	0.22	100	1 fruit	0.22
	Beans	0.71	120		0.85
	Meat, chicken	0.38	100		0.38
	Green vegetable	1	75		0.75
	Passion fruit	0.72	60		0.43
	Papaya	0.89	150		1.34
	Sum				9.75

Vegetables: 2.47, grains (millet): 2.13, fruit: 1.77, legumes: 1.7, milk: 0.67, tea: 0.63.

high antioxidant activity. Since we have no data on finger millet from Uganda, we have used the FRAP value of finger millet from Mali (Halvorsen et al., 2002).

3.7. Effect of food processing

We have mainly analysed the raw fruits and vegetables, except for *Canarium schweinfurthii*, where we analysed both raw and boiled fruits, and found that AA declined from 45.3 to 5.31 mmol $100\ g^{-1}$ when fruits were boiled. For many other vegetables, AA increased when they were boiled (Halvorsen et al., 2006; Aoyama & Yamamoto, 2007; Potsędek, 2007). This study should be followed up by analysing more of the vegetables that are eaten boiled for antioxidant activity in the processed state.

4. Conclusion

The traditional Ugandan diets provide low levels of AA, and they should be improved by adding more fruits and vegetables to increase variation in the different diets. Several traditional dietary plants that can decrease mineral and vitamin deficiency are also high in antioxidants (green leafy vegetables, mangoes, papaya, sweet potato, groundnuts, sesame seeds and finger millet). These have the potential to prevent malnutrition and combat infectious and chronic diseases. Two fruits analysed in this study (raw pulp of *Canarium schweinfurthii* and seed of *Syzygium cuminii*) had very high antioxidant activity and should be further investigated. Malnutrition is a complex problem connected with poverty but, to fight both, information and focused efforts are needed. More research and campaigns on health-related compounds in dietary plants may help to improve the health situation in Africa in a reasonable and sustainable way.

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References

Abayeh, O. J., Abdulrazaq, A. K., & Olaogun, R. (1999). Quality characteristics of *Canarium schweinfurthii* Engl Oil. *Plant Foods for Human Nutrition, 54*, 43–48.

Aoyama, S., & Yamamoto, Y. (2007). Antioxidant activity and flavonoids content of Welch onion (*Allium fistulosum*) and the effect of thermal treatment. *Food Science and Technology Research, 13*(1), 67–72.

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power" The FRAP Assay. *Analytical Biochemistry, 239*, 70–76.

Blomhoff, R., Carlsen, M. H., Andersen, L. F., & Jacobs, D. R. Jr., (2006). Health benefits of nuts: Potential role of antioxidants. *British Journal of Nutrition, 96*(Suppl. 2), S52–S60.

Chetan, S., & Malleshi, N. G. (2007). Finger millet polyphenols: Optimization of extraction and the effect of pH on their stability. *Food Chemistry, 105*, 862–870.

CIA, 2008. World Fact Book. http://www.cia.gov/library/publications/the-world-factbook/print/ug.htlm accessed 08.05.2008.

Dragsted, L. O., Krath, B., Ravn-Haren, G., Vogel, U. B., Vinggaard, A. M., Jensen, P. B., et al. (2006). Biological effects of fruits and vegetables. *Proceedings of the Nutrition Society, 65*, 61–67.

Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I., Hvattum, E., Remberg, S. F., et al. (2002). A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition, 132*, 461–471.

Halvorsen, B. L., Carlsen, M. H., Phillips, K. M., Holte, K., Jacobs, D. R., Jr., & Blomhoff, R. (2006). Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. *American Journal of Clinical Nutrition, 84*, 95–135.

Hegde, P. S., Rajasekaran, N. S., & Chandra, T. S. (2005). Effect of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced rats. *Nutrition Research, 25*, 1109–1120.

Kiguli-Malwadde, E., & Kasozi, H. (2002). Diverticular disease of the colon in Kampala, Uganda. *African Health Sciences, 2*(1), 29–32.

Kulkarni, A. P., & Aradhya, S. M. (2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry, 93*, 319–324.

Lansky, E. P., & Newman, R. A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology, 109*, 177–206.

Navarro, J. M., Flores, P., Garrido, C., & Martinez, V. (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry, 96*, 66–73.

Papaharalambus, C. A., & Griendling, K. K. (2007). Basic mechanisms of oxidative stress and reactive oxygen species in cardiovascular injury. *Trends in Cardiovascular Medicine, 17*, 48–54.

Pellegrini, N., Salvatore, S., Valtueña, S., Bedogni, G., Porrine, M., Pala, V., et al. (2007). Development and validation of a food frequency questionnaire for the assessment of dietary total antioxidant capacity. *The Journal of Nutrition, 137*, 93–98.

Potsędek, A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT, 40*, 1–11.

Rajasekaran, N. S., Nithya, M., Rose, C., & Chandra, T. S. (2004). The effect of finger millet feeding on the early responses during the process of wound healing in diabetic rats. *Biochimica et Biophysica Acta, 1689*, 190–201.

Raschke, V., & Cheema, B. (2008). Colonisation, the new world order, and the eradication of traditional food habits in East Africa: Historical perspective on the nutrition transition. *Public Health Nutrition, 11*, 662–674.

Remberg, S. F., Haffner, K., & Blomhoff, R. (2003). Total antioxidant capacity and other quality criteria in blueberries cvs 'Bluecrop', 'Hardyblue', 'Patriot', 'Putte' and 'Aron' after storage in cold store and controlled atmosphere. *Acta Horticulturae, 600*, 595–598.

Saura-Calixto, F., & Goñi, I. (2006). Antioxidant capacity of the Spanish Mediterranean diet. *Food Chemistry, 94*, 442–447.

Stevenson, D. E., & Hurst, R. D. (2007). Polyphenolic phytochemicals–just antioxidants or much more? *Cellular and Molecular Life Sciences, 64*(22), 2900–2916.

Surveswaran, S., Cai, Y., Corke, H., & Sun, M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chemistry, 102*, 938–953.

Svilaas, A., Sahki, A. K., Andersen, L. F., Svilaas, T., Ström, E. C., Jacobs, D. R. Jr.,, et al. (2004). Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. *Journal of Nutrition, 134*, 562–567.

Thiégang, C., Leudeu, B. C. T., Kapseu, C., Fomethe, A., & Parmentier, M. (2004). Influence of water content of the pulp on the extraction and the physiochemical properties of *Canarium schweinfurthii* oil. *European Journal of Lipid Science Technology, 106*, 613–620.

Totin, D., Ndugwa, C., Mmiro, F., Perry, R. T., Jackson, J. B., & Semba, R. D. (2002). Iron deficiency anemia is highly prevalent among human immunodeficiency virus-infected and uninfected infants in uganda. *The Journal of Nutrition, 132*, 423–429.

UBOS, Uganda Bureau of Statistics (2006). Demographic and Health Survey. Calverton, Maryland, USA.

USDA (2002). National Nutrient Database for Standard Reference. http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl (accessed 25.09.2007).

Veda, S., Platel, K., & Srinivasan, K. (2007). Varietal Differences in the Bioaccessibility of β-Carotene from Mango (*Mangifera indica*) and Papaya (*Carica papaya*) Fruits. *Agricultural and Food Chemistry, 55*(19), 7931–7935.

WHO (2003) Fact sheet No. 134, Traditional Medicine, Revised May 2003. http://www.who.int/mediacentre/factsheets/fs134/en/ Accessed 29.10.2007.

WHO/FAO (2003). Diet, nutrition and the prevention of chronic diseases. http://whqlibdoc.who.int/trs/WHO_TRS_916.pdf, Accessed 09.01.2008.

WHO (2007). Core Health Indicators. http://www.who.int/whosis/database/core/core_select_process.cfm?countries=uga&in. Accessed 11.12.2007.

Paper V

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Full Length Research Paper

Antioxidant and anti-plasmodial activity of extracts from three Ugandan medicinal plants

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Extracts from three plants; *Hallea rubrostipulata, Vernonia adoensis* and *Zanthoxylum chalybeum,* were tested for antioxidant activity using three assays 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Ability of Plasma (FRAP) and total phenol content) and anti-plasmodial activity using an Enzyme-Linked Immunosorbent Assay (ELISA) on *Plasmodium falciparum* chloroquine sensitive strain MRA-285 line. The objective of the study was to find candidates for making anti-malarial phytomedicines. The water extract of *H. rubrostipulata* showed very high anti-plasmodial activity (IC_{50}= 1.95 µg/ml) and high antioxidant activity as well. Thirteen other extracts had high anti-plasmodial activity ranging from 2.14 to 3.63 µg/ml (chloroquine IC50= 8 µg/ml). We found high correlation between the different antioxidant assays.

Key words: *Hallea rubrostipulata, Vernonia adoensis, Zanthoxylum chalybeum,* 2,2-Diphenyl-1-picrylhydrazyl, fluorescence recovery after photobleaching, total phenolic compounds.

INTRODUCTION

Malaria is the most lethal parasitic disease in the tropicical areas. In 2008 there was an estimated 243 million cases of malaria worldwide, that lead to approximately 863 000 deaths. Most of them were young children in Sub Saharan Africa (WHO, 2009). There are four species of *Plasmodium* parasites causing the disease in human: *Plasmodium falciparum*, *Plasmodium malariae, Plasmodium ovale and Plasmodium vivax. P. falciparum* can cause cerebral malaria and is the most malignant. The parasites are usually transferred to humans by *Anopheles gambiae* female mosquitoes. The parasites multiply in the liver and later in the erythrocytes in the blood. Malaria has been very difficult to fight in Africa due to the efficient mosquito, *A. gambiae,* transferring the parasites, a high prevalence of *P.falciparum,* the most deadly species of the parasite vectors, weak infrastructure, high costs to address the disease, and favourable climatic conditions to the vector (Center for Disease Control and Prevention, 2009). The problem has been aggravated by development of resistance by *P. falciparum* towards the most familiar and affordable medicine used for malaria, that is chloroquine.

Anaemia is an important cause of morbidity and mortality in children with acute *P. falciparum* malaria. *Plasmodium*-infected erythrocytes are under constant oxidative stress (Griffiths et al., 2001). In a study of blood from Kenyan children with complicated malaria and malarial anaemia it was found that mean membrane α-tocopherol was significantly reduced and there was a positive correlation between membrane α-tocopherol and haemoglobin concentrations in malarial subjects compared to control. However, no significant difference in plasma α-tocopherol was found. This suggests that in

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severe malaria the red blood cell membrane is exposed to a local increase in oxidative stress, and the erythrocyte's resistance to destruction is linked to the erythrocyte membrane α-tocopherol reserve. It is suggested that compounds exhibiting both anti-plasmodial and antioxidant activities could be very interesting as leads for new anti-malarial drugs (Griffiths et al., 2001; Nguela et al., 2006). In a study of *Plasmodium yoelii* infection in mice oxidative stress and apoptosis (programmed cell death) of liver was demonstrated (Guha et al., 2006) and ·OH was shown to be the causative agent. Since application of radical scavengers protects the liver from oxidative stress Guha et al. (2006) recommend a combination therapy consisting of antioxidants against the ·OH and an antimalarial drug. On the other hand some currently used drugs like artemisinin and methylene blue are believed to act by increasing oxidative stress in the parasites (Becker et al., 2004).

Medicinal plants are still widely used in Uganda to treat malaria (Katura et al., 2007; Ssegawa and Kasenene, 2007; Tabuti, 2008). Some of the plants have been tested for anti-plasmodial activity (Waako et al., 2005; Katuura et al., 2007; Waako et al., 2007). In the West African country Mali an Improved Traditional Medicine (ITM) to treat malaria consisting of three indigenous medicinal plants have been developed (Diallo et al., 2004).

In this study we screened some plants that are commonly used traditionally to treat malaria in Uganda, but where the antioxidant properties of the plants have been poorly investigated. The plants selected for the study included: *Hallea rubrostipulata* (Schum.) J.-F. Leroy (syn: *Mitragyna rubrostipulata*), *Vernonia adoensis* Sch. Bip. ex Walp. (syn: *V. kotschyana* and *Baccharoides adoensis*) and *Zanthoxylum chalybeum* Engl. (syn: *Fagara chalybea*). Flora of Tropical East Africa was used for nomenclature (Beentje, 2000; Bridson and Vercourt, 1988; Kokwaro, 1982). We analyzed the most commonly used plant parts by the respondents in the previous studies (Katura et al., 2007; Ssegawa and Kasenene, 2007; Tabuti, 2008). These included the stem bark of *H. rubrostipulata* and *Z. chalybeum* and the leaves of *V. adoensis.*

Alkaloids have previously been isolated from *H. rubrostipulata* (Seaton et al., 1960; Shellard et al., 1977) and *Z. chalybeum* (Kato et al., 1996). *Z. chalybeum* has been found to exhibit good anti-bacterial (Matu and van Staden, 2003), anti-fungal (Hamza et al., 2006) and anti-plasmodial (Gessler et al., 1994; Rukunga et al., 2009 Muganga et al., 2010; Nguta et al., 2010) activities, while only minimal anti-plasmodial effect was found in *H. rubrostipulata* (Muganga et al., 2010). In several studies on *Z. chalybeum* both root and stem bark where tested and most often the root bark was found to be more active. The stem bark of *Z. chalybeum* is also found to have high antioxidant activity (Kuglerova et al., 2008). The root of *V. adoensis* contains polysaccharides with immunomodulating activity (Nergard et al., 2004) and steroidal saponins and a glycoside (Sanogo et al., 1998) have been identified. In Mali an ITM to treat gastric ulcer, 'Gastrosedal', has now been developed from the powdered roots (Nergard et al., 2004). Extracts of leaves were found to have antimicrobial (Deeni and Hussain, 1994), larvicidal and molluscicidal activity (Diallo et al., 2001). In this study we have made five extracts: acetone, dichloromethane, methanol, CMW (chloroform: methanol: water [12:5:3]), and water, from each of the above plant species. The extracts have been tested for radical scavenger effect by the Diphenyl-1-picrylhydrazyl (DPPH) assay, antioxidant activity by the Ferric Reducing Ability of Plasma (FRAP) method and total content of phenolic compounds using the Folin-Ciocalteu reagent. Different Thin Layer Chromatography (TLC) methods to investigate the presence of main secondary compounds (alkaloids, flavonoids, saponins and bitter principles) were carried out, as well as Nuclear Magnetic Resonance (NMR) of the crude extracts. Anti-plasmodium effect was tested using an Enzyme-Linked Immunosorbent Assay (ELISA) that quantifies parasite histidine-rich protein-2 (HRP-2) (40).

Several studies have demonstrated increased oxidative stress in plasmodium infected red blood cells and liver cells. High anti-plasmodial activity has lately been demonstrated in extracts from *Z. chalybeum.* We believe that combination therapies with plant extracts high in antioxidant activity together with a compound that more specifically target and harm the *P. falciparum* parasites would be good candidates for future antimalarial design. In this study we wanted to detect antioxidant activity in the extracts and if there are correlation between the different antioxidant assays; basic properties in the raw extracts, and investigate if we can find better anti-plasmodial activity in some of the extracts.

MATERIALS AND METHODS

Plant materials

The bark of *H. rubrostipulata* was collected from Sango Bay in Rakai district, leaf material of *V. adoensis* from Mbarara District, while the bark of *Z. chalybeum* was collected in Nakosongola District north of Kampala, all in Uganda. The plant samples for laboratory analysis were air dried in shade to constant weight. Voucher specimen is deposited in the Herbarium of Makerere University (MHU) and at the Norwegian University of Life Sciences (NLH). The plant parts were ground on a laboratory mill, and sieved through a fine meshed copper sieve (0.250 microns mesh width).

Extraction of plant materials

For each plant 1 g finely ground plant material was extracted with 10 ml solvent in a centrifuge tube. The extract was decanted after centrifugation at 1800 x g for 5 min at 25°C basically as described by Elloff (1998). The process was repeated 3 times. The extractants used were technical grade acetone, Dichloromethane (DCM),

methanol (MeOH), a mixture of methanol, chloroform and water (MCW; 12:5:3) and water. The extracts were concentrated to dryness using a rotavapor with temperature 40°C, and the dried extracts were stored at -20°C until use. They were re-dissolved in water or methanol to prepare solutions of different concentrations for further studies (Figure 1).

Free radical scavenger and antioxidant bioassays DPPH radical scavenging

For testing radical scavenging activity, reaction with the DPPH radical was carried out as previously described (Wangensteen et al., 2004). Extracts were dissolved in methanol, and quercetin (Sigma-Aldrich) was used as a positive control. A Biochrom Libra S32 spectrophotometer (Biochrom, Cambridge, England) was used for measurements of ultraviolet (UV) absorbance at 517 nm.

Antioxidant activity using the ferric reducing ability of plasma method

Antioxidant activity in the samples was measured using the Ferric Reducing Ability of Plasma (FRAP) assay (Benzie and Strain, 1996), using some modification (Halvorsen et al., 2002; Stangeland et al., 2009) where the samples were diluted in methanol instead of water. The FRAP-method measures the absorption change that appears when the TPTZ-Fe^{3+} complex is reduced to TPTZ-Fe^{2+} form in the presence of antioxidants. An intense blue colour with absorption maximum at 593 nm develops. Our measurements were performed at 595 nm with incubation time of 10 minutes at 37 ºC by using the KoneLab 30i spectrophotometer (Kone Instruments Corp., Espoo, Finland). The antioxidant activity in the samples was calculated as µmol Fe^{2+} per gram (µmol/g) extract.

Total phenolic compounds

The total amount of phenolic compounds (TP) of the different extracts was determined using the KoneLab 30i spectrophotometer. The procedure was based on using the Folin-Ciocalteu Reagent (FCR) (Singleton et al., 1999). In brief, 20 µl of extract were added to 100 µl FCR (diluted 1:10 with dist. water), mixed and incubated at 37°C for 60 s, 80 µl of 7.5 % (w/v) sodium bicarbonate solution was added, the samples were again mixed and incubated at 37°C for 15 min prior to absorbance reading at 765 nm. TP was assessed against a calibration curve of gallic acid, and the results presented as mg Gallic Acid Equivalents (GAE) per g extract.

Thin layer chromatography

The extracts were tested in different TLC systems and spray reagents to show the presence of alkaloids, flavonoids, saponins and bitter principles following standard procedures as described by Wagner and Blatt (1996). Components were visualised by UV irradiation (254 and 365 nm) or visual light.

Nuclear magnetic resonance

^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker DPX 300 instrument (Bruker Biospin GmbH, Rheinstetten, Germany). Samples were dissolved in $CDCl_3$, CD_3OD or DMSO d-6, and TMS was used as reference.

***In vitro* anti-plasmodial assay**

Blood stage assays

To test anti-plasmodial activity of the plant extracts the susceptibility micro assay technique was used. *P. falciparum* chloroquine sensitive strain MRA-285 line was maintained in a continuous culture by the method of Trager and Jensen (1976) and used in these assays. The vial of the frozen strain was removed from liquid nitrogen; thawed at 37°C in a water bath for 2 min without shaking. The contents of the vial were transferred into a 50 ml centrifuge tube. 0.1 ml of 12% NaCl solution was added drop-wise to the pellet at a rate of 1 drop per second with constant agitation. 10 ml of 1.6% NaCl was then added drop wise to the pellet at a rate 1 drop per second with constant agitation to achieve complete mixing. The contents were centrifuged at 2000 RPM for 5 min and the supernatant discarded. 10 ml of pre-warmed complete culture medium was added and the mixture transferred to the culture flask. Giemsa-stained thin blood smears were prepared and examined, parasitemia was determined and contents put in a candle jar and incubated at 37°C for 24 h. After incubation the parasitemia of the surviving trophozoites was determined. The contents were span at 2000 RPM for 5 min at RT, supernatant removed and discarded. 10 ml of pre-warmed complete culture medium was added and fresh RBC to 2% hematocrit and parasitemia adjusted to less than 1%. 10 ml RPMI 1640 medium supplemented with 25 mM HEPES, 0.2% NaHCO3, 0.1 mM hypoxanthine, 100 µg/ml gentamicin, and 0.5% Albumax II serum substitute to produce a packed cell volume of ~2% was used.

Measurement of *in vitro* drug sensitivity

Sensitivities were measured for chloroquine diphosphate (CQ) (Sigma-Aldrich) as the positive control, no drug as negative control and the plant crude extracts as test samples. Since chloroform is toxic to the parasites, chloride water mixed with methanol was used instead for the CMW extracts. The measurements were performed as described by Nsobya et al. (2010), except that crude plant extracts instead of drugs were tested. All procedures were performed with a vortex in the hood and the surface was wiped with 70% alcohol in order to obtain aseptic conditions.

Statistical analysis

All assays were analysed in triplicate. Inhibitory concentrations (IC_{50}s) for antimalarials were calculated using a polynomial regression model and HN-NonLin software, which is available at http://malaria.farcH.net. Correlations between drugs were evaluated by Pearson correlation. Associations between drug sensitivities and specific parasite polymorphisms were evaluated using the Fisher exact test. For all statistical tests, the significance level was set at $P < 0.05$. Correlations between the different antioxidant and radical scavenger assays were run in Minitab and the Pearson correlation was calculated.

RESULTS AND DISCUSSION

The acetone extract of *H. rubrostipulata* had highest antioxidant activity with IC_{50} DPPH 16.6 µg/ml, FRAP 5370.5 µmol/g and total phenols 370.7 mg GAE/g (Table 1, Figure 2). Methanol, Chloroform, Methanol, Water (CMW) and water extract of both *H. rubrostipulata* and *Z. chalybeum* showed quite high activity ranging from 23.9 to 46 µg/ml DPPH, 2478.3 to 4167.5 µmol/g extract

Table 1. Yield, DPPH radical scavenging, FRAP antioxidant activity and total phenolics of *H. rubrostipulata, Z. chalybeum and V. adoensis*.

Scientific name Family Local name[a]	**Collection** No.	**Part** used[b]	Solvent[c]	**Sample** ID	**Yield** %[d]	**DPPH** IC-50 ± SD	**FRAP** µmol/g extr.±SD	**Total phenolics** GAE/g extr.±SD
H. rubrostipulata	TS 104	Sb	Aceton	H-Ac	9	16.6 ± 0.5	5370.5 ± 28.1	370.7 ± 0.25
Rubiaceae			DCM	H-DCM	1	>167	253.8 ± 5.4	26.4 ± 0.09
Muziku (GA)			Methanol	H-Me	11	46 ± 2.1	2562.6 ± 9.6	187.6 ± 0.91
			CMW	H-CMW	5	23.9 ± 0.6	3633.1 ± 6.6	269.5 ± 0.48
			Water	H-W	12	28.7 ± 0.9	2863.5 ± 11.0	207.9 ± 0.30
V. adoensis	TS 338	L	Aceton	V-Ac	9	>167	502.0 ± 2.9	32.0 ± 0.11
Asteraceae			DCM	V-DCM	9	>167	193.2 ± 2.1	14.6 ±0.10
Nyakayuma (NYA)			Methanol	V-Me	9	39.6 ± 3.7	2311.6 ± 5.1	163.1 ± 1.02
			CMW	V-CMW	8	33.3 ± 1.7	2562.3 ± 25.0	186.2 ± 0.25
			Water	V-W	20	53.9 ± 2.5	1474.7 ± 7.9	98.1 ± 0.17
Z. chalybeum	TS 345	Sb	Aceton	Z-Ac	5	80.9 ± 8.7	1663.2 ± 8.8	134.6 ± 0.79
Rutaceae			DCM	Z-DCM	4	>167	184.7 ± 1.6	28.8 ± 0.07
Ntaleyedungu (GA)			Methanol	Z-Me	26	25.2 ± 0.7	4167.5 ± 3.0	333.5 ± 1.40
			CMW	Z-CMW	21	26.7 ± 1.2	3644.4 ± 6.8	286.1 ± 0.28
			Water	Z-W	26	44.4 ± 2.7	2478.3 ± 10.2	233.0 ± 3.32

[a] Local name: GA, Luganda; NYA, Runyankole, [b] Sb, stem bark, L, leaves, [c] DCM, dichloromethane; CMW, chloroform, methanol, water (12:5:3), [d] w/w yield in terms of initial dried material.

FRAP and 187.6 to 333.5 mg GAE/g extract total phenols. The strongest anti-plasmodial activity was found in the water extract of *H. rubrostipulata* (Table 3) (IC_{50} = 1.95 µg/ml) which is regarded as very high activity (Gessler et al., 1994). Vernonia adoensis has not previously been tested for anti-plasmodial activity. We found that all extracts from V. adoensis had very high anti-plasmodial activity ranging from 2.14 – 2.83. Four of the Z. chalybeum extracts demonstrated high activity as well (IC50 ranging from 2.72 – 3.94 µg/ml); only the methanol extract had low activity (IC50= 10.92 µg/ml). Chloroquine control (CQ sensitive strain MRA-285) showed IC_{50} = 8 µg/ml.

The DPPH radical scavenging activity is due to the hydrogen-donating ability, while the FRAP assay measures the extract's ability to reduce iron (Fe^{+++}). The total amount determined to be of phenolic nature by the FCR is based on the chemical reduction of the FCR, a mixture of tungsten and molybdenum oxides, resulting in blue coloured products with absorption maximum 765 nm. The intensity of the absorption is equivalent to the sum of the individual contribution of the different classes of phenols in the mixture (Singleton et al., 1999). Other readily oxidised substances might also react with the reagent. The results show high correlation between the three methods used (Table 1 and Figure 2), and Pearson correlation between the three assays varied between 0.945 and 0.988, all with a P-value of =0.000. In Figure 2 the inverse value of DPPH is used, since in this assay the lowest value show the highest activity.

The DCM extract of all species had the lowest activity, but this solvent also gave low yield (Table 1). Very few studies of antioxidant and radical scavenger effect of extracts from these plants were found. However our results for the DPPH test of methanol extract of *Z. chalybeum* is close to results found by Kuglerova et al. (2008) for ethanol extracts, with IC_{50} values of 25.2 and 23 µg/ml respectively. Diallo et al. (2001) found weak radical scavenger effect in the DCM extract of *V. adoensis* leaves, while no activity in the same extract was found in this study (Table 1). In our study the strongest antioxidant activity when measured with the FRAP assay had H-Ac > Z-Me > Z-CMW > H-CMW > H-W > H-Me > V-CMW.

The selected plants were extracted with five different extract systems (Table 1), and the water extract gave highest yield for all plants. The ^{1}H and ^{13}C NMR spectra of the water extracts indicated high amounts of sugar, in addition content of compounds with aromatic and olefinic structures in all water extracts was observed. The presence of alkaloids, flavonoids, saponins and bitter principles in the different extracts is shown in Table 2. Alkaloids were found in all three plants as demonstrated earlier (Seaton et al., 1960; Shellard et al., 1977; Kato et al., 1996; Gessler et al., 1994; Deeni and Hussain, 1994), but not in the DCM extract of *H. rubrostipulata* and water extract of *Z. chalybeum.* Flavonoids, saponins and bitter principles were present in most extracts, except for the DCM extracts of *H. rubrostipulata* and *Z. chalybeum* which were nearly deficient of NMR signals in the aromatic region as well. The acetone extract of *V. adoensis* had no saponins or bitter principles.

In a screening of antimalarial activity in 58 plant samples from 43 species of medicinal plants from Tanzania, Gessler et al. (1994) found that extract from *Z. chalybeum* root bark (ethanol and water) were among the four top plant parts with IC_{50} values < 1 µg/ml (hypoxanthine assay using the multidrug resistant *P.*

Table 2. Phytochemical screening of crude extracts.

Sample ID	Alkaloids	Flavonoids	Saponins	Bitter principles
H-Ac	+	+	+	+
H-DCM	-	-	-	-
H-Me	+	+	+	+
H-CMW	+/-	+	+	+/-
H-W	+/-	+	+	+/-
V-Ac	+	+	+/-	-
V-DCM	+	+	+	+/-
V-Me	+/-	+	+	+
V-CMW	+	+	+	+
V-W	+	+	+	+
Z-Ac	+	+	-	+
Z-DCM	+/-	+/-	-	-
Z-Me	+	+	+	+
Z-CMW	+	+	+	+
Z-W	-	+	+	+/-

+/-: indistinct result.

Table 3. Anti-plasmodial activity in extracts from *H. rubrostipulata, V. adoensis* and *Z. chalybeum* with chloroquine as positive control on chloroquine sensitive strains.

Species	Ac		DCM		MeOH		CMW		W	
	% Y	IC_{50}*	% Y	IC_{50}*	% Y	IC_{50}*	% Y	IC_{50}*	% Y	IC_{50}*
H. rubrostipulata	9	2.97	1	2.83	11	3.94	5	2.70	12	1.95
V. adoensis	9	2.54	9	2.83	9	2.57	8	2.67	20	2.14
Z. chalybeum	5	3.05	4	2.85	26	10.92	21	2.72	26	3.63
Chloroquine										8

Ac, Acetone; DCM, Dichloromethane; MeOH, Methanol; CMW, chlorine water and methanol, Y= Yield, W = Water, * µg/ml.

falciparum stain K1, and a chloroquine resistant strain NF54). Five other species had IC_{50} values = 1- 4.9 µg/ml, which was regarded as high activity. All of the three plants in this study had extracts with very high or high activity according to Gessler et al. (1994). In a study of Rwandan medicinal plants (Muganga et al., 2010), both *H. rubrostipulata* and *Z. chalybeum* were among 13 plants tested for anti-plasmodial activity against a chloroquine-sensitive strain of *P. falciparum* (3D7), and the most active extracts were evaluated against a chloroquine sensitive strain (W2). Root bark of *Z. chalybeum* showed high activity (Me: 4.2 ± 2.7 µg/ml; DCM: 6.2 ± 0.6 µg/ml), while methanol extract of *H. rubrostipulata* only showed slight activity (39.9 ± 2.8 µg/ml). In this study the limit for activity was defined to > 50 µg/ml. However the activity of DCM extract from stem bark of *H. rubrostipulata* and *Z. chalybeum* was quite similar, as it was in our study as well (our study: 2.83 and 2.85 µg/ml; Muganga et al. (2010): 39.9 and 41.5 µg/ml for *H. rubrostipulata* and *Z. chalybeum* respectively). In a study of anti-plasmodial activity in plant extracts traditionally used in Kenya the highest activity was found in *Z. chalybeum* root bark water extract with IC_{50} value of 3.65 µg/ml (Rukunga et al., 2009). There have lately been several promising studies of the anti-plasmodial activity of *Z. chalybeum,* especially the root bark. We have analyzed the stem bark, and found high activity in several of the extracts. In further work both the root and stem bark should be analysed in more detail. *Z. chalybeum* is one of the species heavily harvested both for medicine and for charcoal production, and it has been found difficult to cultivate. The species has been ranked as one of the 35 most important medicinal species of conservation concern in the world (Hawkins, 2008). Measures to multiply and sustain the species should be intensified.

The stem bark water extract of *H. rubrostipulata* was found to have the highest activity against the *P. falciparum* strain tested. This extract also had relative high score in the antioxidant tests (DPPH IC_{50}: 28.7 µg/ml, FRAP: 2863.5 µmol/g, TP: 207.9 mg GAE/g), and should be investigated further for potential development of phytomedicines. *H. rubrostipulata* has been found easy to germinate and grow (Stangeland et al., 2008) as opposed to *Z. chalybeum,* and it will thus be possible to secure raw material for potential phytomedicines.

We found that there is a strong correlation between the different antioxidant assays(Figure 2), and many of the extracts showed high anti-plasmodial activity. Some of the with high anti-plasmodial activity also have a extracts

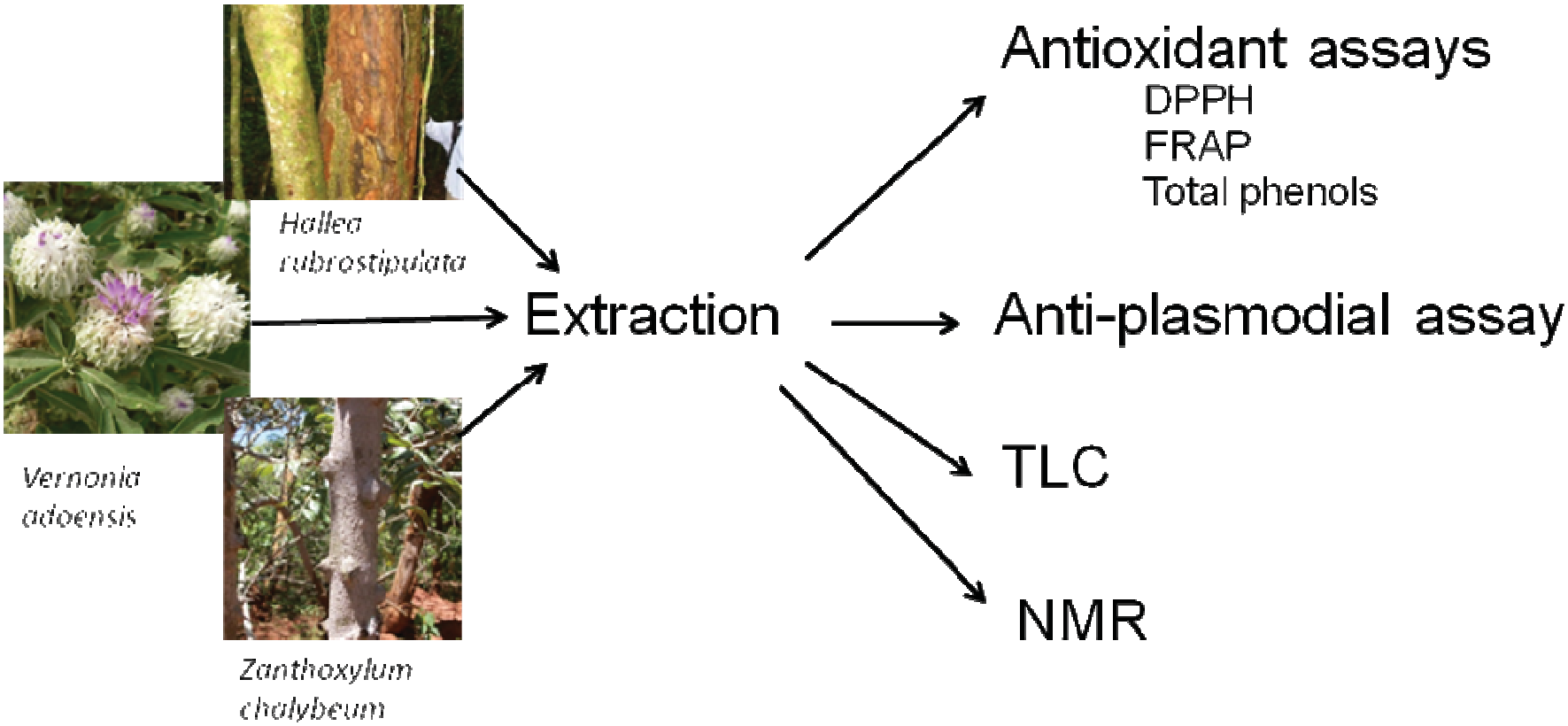

Figure 1. Picture of the three plants analyzed in this study. The arrows show the extraction process and different assays conducted.

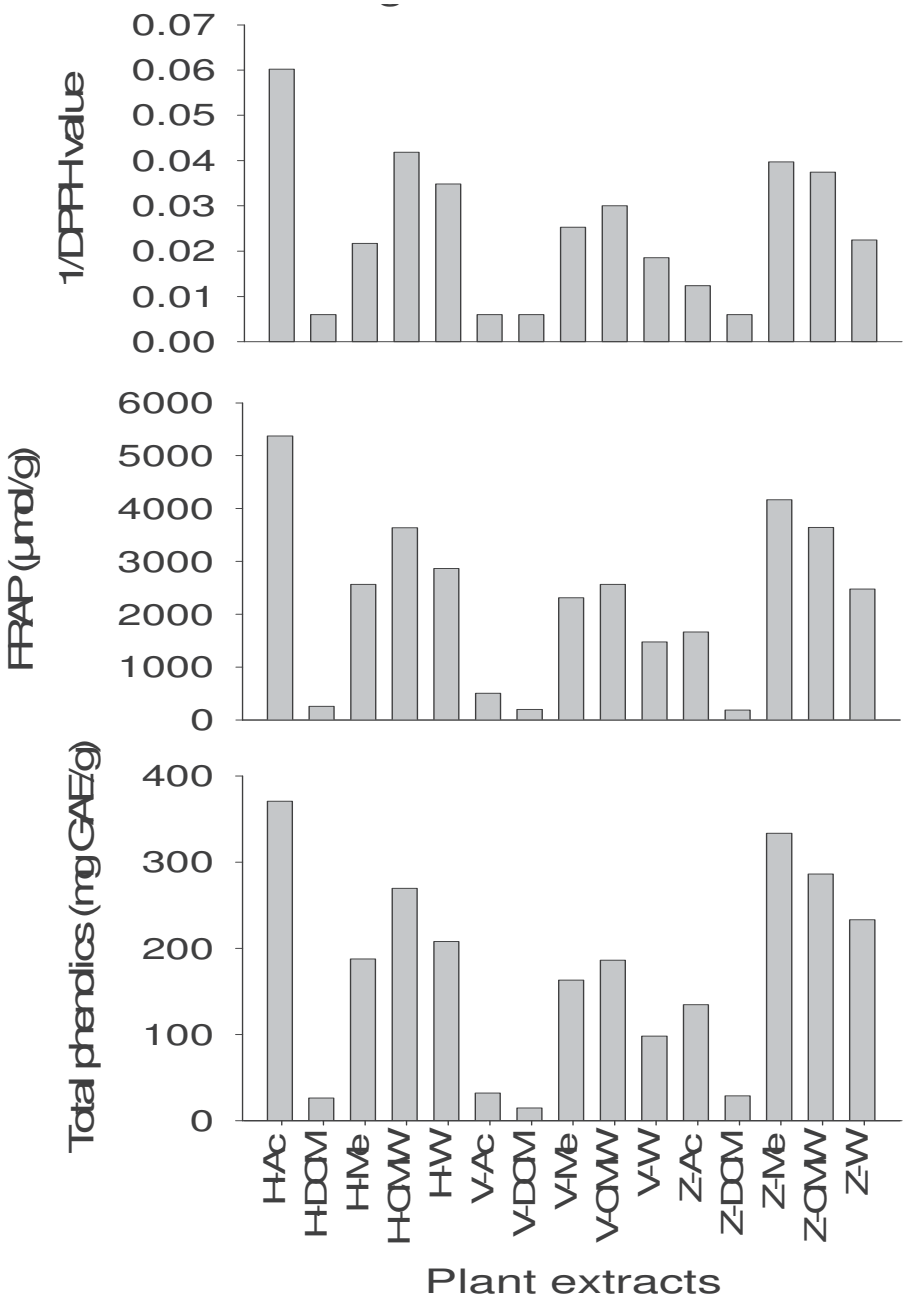

Figure 2. The inverse value of DPPH IC_{50} radical scavenger activity (top), antioxidant activity measured by the FRAP assay (middle) and total phenols measured by the Folin-Ciocalteu assay (bottom) in plant extracts from *H. rubrostipulata, V. adoensis* and *Z. chalybeum*. For plant extract sample IDs see Table 1.

Rather high antioxidant activity, like the water extract of all the three species and the methanol extracts of *H.rubrostipulata* and *V. adoensis.* According to Nguela et al. (2006) these extracts could be interesting leads to look for good anti-malarial drugs. Gessler et al. (1994), Muganga et al. (2010), and Rukunga et al. (2010) all found very high *in vitro* anti-plasmodial activity in some of the extracts from *Z. chalybeum* root bark, and moderate activity in the stem bark. This is in line with our findings for activity in stem bark. Muganga et al. (2010) only found moderate anti-plasmodial activity in the *Hallea* (*Mitragyna) rubrostipulata* stem bark*,* but they did not test the water extract of *M. rubrostipulata,* where we found the highest activity. According to previous studies and

our findings,stem bark and root bark of *Z. chalybeum* and *M. rubrostipulata* are good candidates for further investigation. *In vitro* assays cannot precisely reproduce *in vivo* situation. Some extracts may not become active without certain metabolic action *in vivo.* In further work the extracts should be tested with *Plasmodium berghei* in mice and toxicological investigations.

Conclusion

During malaria infection increased reactive oxygen species are generated that may contribute to erythrocytic damage and anaemia. On the other hand some hypothesise that important antimalarials act as pro oxidants in the plasmodium parasite. In any case, oxidative stress seems to play an important role in erythrocytes and parasites during malaria infection. In our bioassays many plant extracts showed high anti-plasmodial as well as high antioxidant activity. We do not know if it is the same or different compounds in the raw extracts that have these effects, and neither do we know at this stage the bioavailability of the active principles. But since the water extracts of these plants have been traditionally used against malaria there is reason to believe that there is some bioavailability. We believe that it may be favourable to select extracts with high anti-plasmodial and antioxidant activity for further investigation. In future work the most promising raw extracts, like the water extract of *H. rubrostipulata* and *V. adoensis,* should be tested both on chloroquine sensitive and chloroquine resistant strains and bioassay guided fractionation for antimalarial effect and toxicity should be performed to see if effective and safe antimalarials can be developed.

REFERENCES

Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H (2004). Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. Int. J. Parasitology, 34:163-189.

Beentje HJ (2000). Flora of Tropical East Africa, Composita Part 1, Edited by Beentje HJ, Smith SAL. Rotterdam: A.A. Balkema, Rotterdam.

Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power" The FRAP Assay. Anal. Biochem. 239:70-76.

Bridson D, Vercourt B (1988). Flora of Tropical East Africa, Rubiaceae Part 2, Edited by Polhill RM, A.A. Balkema, Rotterdam.

Centre for Disease Control and Prevention. Malaria. http://www.cdc.gov/malaria/about/index.html

Deeni YY, Hussain HSN (1994). Screening of *Vernonia kotschyana* for Antimicrobial Activity and Alkaloids. Pharm. Biol. 32:388-395.

Diallo D, Marston A, Terreaux C, Toure Y, Paulsen BS, Hostettmann K (2001). Screening of Malian plants for antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging activities. Phytother. Res. 2001, 5:401-406.

Diallo D, Maïga A, Diakité C, Willcox H (2004). Malarial-5: Development of an Antimalarial Phytomedicine in Mali. In: Willcox. (eds) Traditional Medicinal Plants and Malaria . CRC Press, Boca Raton, pp 117-130.

Elloff JN (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol. 1998, 60:1-8.

Gessler HC, Nkunya HHH, Mwasumbi LB, Heinrich H, Tanner H (1994). Screening Tanzanian Medicinal Plants for Antimalarial activity. Acta. Trop. 56:65-77.

Griffiths HJ, Ndungu F, Baird KL, Muller DPR, Marsh K, Newton CRJC (2001). Oxidative stress and erythrocyte damage in Kenyan children with severe *Plasmodium falciparum* malaria. Br. J. Haematology 113:486-491.

Guha H, Kumar S, Choubyey V, Maity P, Bandyopadhyay U (2006). Apoptosis in liver during malaria: role of oxidative stress and implication of mitochondrial pathway. FASEB J. 20:E439-E449.

Halvorsen BL, Holte K, Myhrstad HCW, Barikmo I, Hvattum E, Remberg SF, Wold A-B, Haffner K, Baugerød H, Andersen LF, Moskhaug JØ, Jacobs Jr. DR, Blomhoff R (2002). A systematic screening of total antioxidants in dietary plants. J. Nutr. 132:461-471.

Hamza OJH, van den Beukel CJP, Matee HIN, Moshi HJ, Mikx FHH, H.O. Selemani HO, Mbwambo ZH, van der Ven AJAH, Verweij PE (2006). Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. J. Ethnopharmacol. 2006, 108:124-132.

Hawkins B (2008). *Plants for life: Medicinal plant conservation and botanic gardens.* Botanic Gardens Conservation International. Richmond.

Kato A, Moriyasu H, Ichimaru H, Nishiyama Y, Yuma FD, Nganga JN, Mathenge SG, Ogeto JO (1996). Isolation of alkaloidal constituents of *Zanthoxylum umbarence and Zanthoxylum chalybeum* using ion-pair HPLC. J. Nat. Prod. 59:316-318.

Katuura E, Waako P, Ogwal-Okeng J, Bukenya-Ziraba R (2007). Traditional treatment of malaria in Mbarara District, western Uganda. Afr. J. Ecol. 45:48-51.

Katuura E, Waako P, Tabuti JRS, Bukenya-Ziraba R, Ogwal-Okeng J (2007). Antiplasmodial activity of extracts of selected plants used by local communities in Western Uganda for treatment of malaria. Afr. J. Ecol. 45:94-98.

Kokwaro JO (1982). Flora of Tropical East Africa, Rutaceae. Edited by Polhill RM. A.A. Balkema, Rotterdam.

Kuglerova H, Marsik P, Vanek T, Kokoska L, Wanyana-Maganyi O, Van Damme P, Grade J (2008). Antioxidative activity of Ugandan Medicinal Plants [abstract]. Afr. J. Trad. Compl. Alt. Medicines, Cape Town. http://journals.sfu.ca/africanem/index.php/ajtcam/article/view/745 , assessed 07.04.2010.

Matu EN, van Staden J (2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. J. Ethnopharmacol. 87:35-41.

Muganga R, Angenot L, Tits M, Frédérich M (2010). Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in treatment of malaria. J. Ethnopharmacol. 128:52-57.

Nergard CS, Diallo D, Michaelsen TE, Malterud KA, Kiyohara H, Matsumoto T, Yamada H, Paulsen BS (2004). Isolation, partial characterisation and immunomodulating activities of polysaccharides from *Vernonia kotschyana* Sch. Bip. ex Walp. J. Ethnopharmacol. 91:141-152.

Ngouela S, Lenta BN, Noungoue DT, Ngoupayo J, Boyom FF, Tsamo E, Gut J, Rosenthal PJ, Connolly JD (2006). Anti-plasmodial and antioxidant activities of constituents of the seed shells of *Symphonia globulifera* Linn. f.. Phytochemistry, 67:302-306.(2005).Guha H, Kumar S, Choubey V, Maity P, Bandyopadhyay U (2006). Apoptosis in liver during malaria: role of oxidative stress and implication of mitochondrial pathway. FASEB J. 20:E439-E449.

Nguta JH, Mbaria JH, Gakuya DW, Gathumbi PK, Kiama SG (2010). Antimalarial herbal remedies of Msambweni, Kenya. J. Ethnopharmacol. 128:424-432.

Nsobya SL, Kiggundu H, Nanyunja S, Joloba H, Greenhouse B, Rosenthal PJ (2010). In vitro sensitivities of *Plasmodium falsiparum* to different antimalarial drugs in Uganda. Antimicrob. Agents Chemother. 54:1200-1206.

Rukunga GH, Gathirwa JW, Omar SA, Muregi FW, Muthaura CN, Kirira PG, Mungai GH, Kofi-Tsekpo WH (2009). Anti-plasmodial activity of the extracts of some Kenyan medicinal plants. J. Ethnopharmacol. 121:282-285.

Sanogo R, Germano MP, de Tommasi N, Pizza C, Aquino R (1998). Vernoniosides and an androstane glycoside from *Vernonia kotschyana.* Phytochemistry 47:73-78.
Seaton JC, Nair HD, Edwards OE, Marion L (1960). Structure and stereoisomerism of three mitragyna alkaloids. Can. J. Chem. 38:1035-1042.
Shellard EJ, Houghton PJ, Lala PK (1977). Rotundifoline *N*-oxides. Phytochemistry 16: 1427-1429.
Singleton VL, Orthofer R, Lamuela-Raventos RH (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299:152-178.
Ssegawa P, Kasenene JH (2007). Plants for malaria treatment in Southern Uganda: Traditional use, preference and ecological viability. J. Ethnopharmacol. 27:110-131.
Stangeland T, Tabuti JRS, Lye KA (2008). The influence of light and temperature on the germination of two Ugandan medicinal trees. Afr. J. Ecol. 46:565-571.
Stangeland T, Remberg SF, Lye KA (2009). Total antioxidant activity in 35 Ugandan fruits and vegetables. Food Chem. 113:85-91.
Tabuti JRS (2008). Herbal medicines used in the treatment of malaria in Budiope County, Uganda. J. Ethnopharmacol. 116:33-42.
Trager W, Jensen JB (1976). Human malaria parasites in continuous culture. Science 193:673-675.
Waako PJ, Gumede B, Smith P, Folb PI (2005). The in vitro and in vivo antimalarial activity of *Cardiospermum halicacabum* L. and *Momordica foetida* SchumcH. Et Thonn.. J. Ethnopharmacol. 99:137-143.
Waako PE, Katuura E, Smith P, Folb PE (2007). East African medicinal plants as a source of lead compounds for the development of new antimalarial drugs. Afr. J. Ecol. 45:102-106.
Wagner H, Blatt S (1996). Plant drug analysis. A Thin Layer Chromatography Atlas, 2nd ed., Springer, Berlin.
Wangensteen H, Samuelsen AB, Malterud KE (2004). Antioxidant activity in extracts from coriander. Food Chem. 88:293-297.
World Health Organisation. World Malaria Report 2009. http://www.who.int/malaria/world_malaria_report_2009/en/index.html.

Paper VI

Plants used to treat malaria in Nyakayojo sub-county, Western Uganda

Torunn Stangeland

1. Introduction

Malaria is the single most important cause of ill health, mortality and poverty in Africa South of Sahara. The disease has become more severe as the parasite causing most of the infections in Africa, *Plasmodium falciparum,* has developed resistance towards the most common and affordable medicines to treat malaria. People with reduced immune system like pregnant women and children below five years of age are the groups most vulnerable to malaria. Around 1 million people die from malaria each year, and most of them are young African children (WHO 2009). Some studies indicate that malnutrition; iron, vitamin A and zinc deficiency is connected to increased malaria mortality, still births and underweight at birth (Caulfield et al. 2004, Lartey 2008). A few African countries like Rwanda, Zambia, Eritrea and Zanzibar have managed to reduce recorded deaths caused by malaria by 50% due to adequate coverage of key interventions like insecticide treated bed nets and intensified treatment of cases. However the international disbursement to malaria-endemic countries is still far from what has been promised and is needed according to WHO (2009). This situation will probably worsen because of the financial crisis. The G8 meeting in Toronto in June 2010 between the 8 richest countries in the world admitted that they will come up 30% short on a pledge made in 2005 on raising their combined fundraising for the poorest countries (Wroughton 2010). However they discussed how to target their efforts and one of the targets is improving mother-and-child health. In order to make Africa less vulnerable to international problems, it is important to find solutions that build on the local knowledge and resources. In East Africa there is a long tradition of treating disease with medicinal plants, and this knowledge is still practiced, although the trust in the tradition is in some cases decreasing (Tabuti 2008), as is the knowledge. However in many rural areas the Health Centers are poorly equipped both with personnel and medicines, so people have to use what is available. In this situation WHO (2008) urges developing countries to include TM in their primary health care, but it is important to find out which of the traditional medicinal plants and treatments that are effective and safe to use.

Most villages have a traditional birth attendant (TBA) and often several traditional healers (THs), and if health is going to be improved cooperation between everyone in the villages, which are concerned with people's health, is necessary. In Nyakayojo sub-county we cooperated with a group of traditional birth attendants and healers, and the local health

centers (HC) in 2007. We have had three workshops with reproductive health care and income generating activities on the program. The workshops were held at a Health Center, and there was always some of the HC staff present. We urged the TBAs to cooperate with the HC, and to report deliveries. We decided to use this group as informants for our interviews, and they very generously shared their knowledge and recipes with us. At the concluding workshop we asked the HC personnel if they were willing to help us with a clinical trial to test one or two of the recipes. They agreed to perform blood smear tests on people that preferred to use herbal treatment on uncomplicated malaria before and after the treatment. Until now we have missed funding and time to accomplish this project, but we still hope for the possibility to do so later.

In this study we wanted to gather detailed information about how the TBAs and THs use plants to treat malaria. Azas et al. (2002) found synergistic in vitro antimalarial activity in a mixture of plants traditionally used. Further some use fresh juice from plant parts, while others make decoctions, and it is important to find what mode of preparation is the most effective.

2. Material and method

Mbarara District is located in Western Uganda 270 km southwest of Kampala. The fieldwork was mainly done in Nyakayojo sub-county (0º63´S and 30.61ºE). The area has a mean annual maximum temperature of 25-27.5 °C, and mean annual rainfall of 900-1000 mm (Anonymous 1967). The area is hilly and the main economic activity is mixed farming, cultivation of crops and grazing cattle and goats. Prior to this study one of the colleagues in the NUFU medicinal plant project had worked with traditional birth attendants (TBAs) to document their use of plants in reproductive health care. The informants are organised in "Nyakayojo Traditional Healers and Traditional Birth Attendants Association" and come from many villages in the sub-county. They are 27 women and one man.

A questionnaire was developed for the purpose, and semi-structured interviews were performed by a group of three persons: a botanist, a pharmacologist and a physician. Pregnant women are the most seriously hit group by malaria next to children, and all of the TBAs knew how to use plants to treat the disease.

Plant specimens were collected, and brought to UMB for identification by Kåre A. Lye. Specimens are deposited at the herbarium at Makerere and Norwegian University of Life Sciences.

3. Results

3.1. Respondents' socioeconomic characteristics

Only one of the respondents was a man. Most of them belong to the Munyankole tribe (Table 1), had none or primary education and mostly belonged to the Christian religion. Their main source of livelihood was farming, and their secondary job was mainly TBA or TH.

3.2. Traditional knowledge about malaria

The understanding of malaria was good. Most of them recognised one or more of the most frequent symptoms of malaria, as high body temperature, shivering, headache, vomiting (Table 2). All symptoms given can be found in a person with malaria. However the understanding of the cause of malaria was not that good. Even if 86 % of the respondents new that mosquito bite cause malaria, as many as 25 % thought that drinking unboiled water can cause malaria (Table 3). Other reasons like dirty environment, worms, transition from bad mattress were also mentioned by some. Some mentioned indirect causes like bad feeding, cold weather (more mosquitoes then), grass around homestead and stagnant water.

3.3. Malaria treatment practices

Some of the informants mainly treated their own family while others had several patients per month. In average 17 patients were treated per month, ranging from 5 patients per year to 200 per month. The latter was nurse assistant at a health centre and used both plants and allopathic treatment of patients there.

They expressed that it has become more difficult to treat malaria; often the disease becomes more severe than earlier. They all refer to Health Centre, if their treatment does not work within a few days, or at once if the symptoms are severe. In that case they may give some first aid plants to take down the fever. One of the TBAs said she never treated with plants unless in cooperation with health personnel.

The information gathered in the interviews revealed that a wide range of plants and recipes were used to treat malaria. Altogether 57 species were registered used, but 47 % were only used by one of the TBAs (Table 4 and 5). *Vernonia amygdalina* Delile was by far the most commonly used plant (86 % of respondents), and mainly the leaves were used. The most common mode of preparation was to squeeze juice out of the leaves and add some cool, pre boiled water. Next to *V. amygdalina* the indigenous *Aloe* sp., *Justicia betonica* T.Anderson and *Vernonia adoensis* Sch.Bip. ex Walp. was the most commonly used plants by the respondents. It was common to use several plants together in a recipe (Table 5), and most of the plants that were only used by one TH or TBA were parts in mixtures. The most common mode of administration was oral, bur also bath and steam baths were used. Leaves were by far the most common plant part, used in 85% of recipes; in addition roots and root bark, flowers and seeds were used. Almost all the respondents had more than one recipe for treating malaria, and many of them said that if the first one did not help within two or three days, they tried another or referred to HC or hospital. One of the TBAs only used one recipe, but this was a combination of all the most commonly used plants (the last of the recipes under *V. amygdalina*). Six of the plants were dried in the shade and stored for use, while leaf of two *Aloe* sp. were added as the decoction boiled. This seems like a cost and time effective solution, and it would be interesting to test the effect of this recipe. Two of the TBAs were growing *Artemisia annua* L. in their garden and informed that they had learned about the plant and got seedlings of a white man a year earlier.

Conclusion

This study shows that there is a great diversity in how a group of traditional birth attendants and healers use plants and modes of preparation to treat malaria, even if they are in the same association. To follow up this study a literature search should be carried out to find how common the use of the plants is other places in Uganda or Africa. If the same plant is used for the same or similar disease in different parts of Uganda or Africa it can indicate good effect of the treatment. There could be a preliminary study following the response of herbal treatment on patients with malaria. One or more treatments should be selected and tested in a clinical trial, and further analysis of the plants used should be performed, if it is not already done. Actually, several plants have proved to have activity against malaria, but a final part is lacking: how to turn the active plants into a phytomedicine which are safe and effective. If this kind of research is going to have effect on the health and well being of people in Africa,

it is of great importance to have time and funding to follow the process until a product is developed. This is possible and has already been done in the West African country of Mali where the Improved Traditional Medicine "Malarial-5" has been developed. With joint efforts we hope a similar development can occur in East Africa as well.

References

Anonymous, 1967. Atlas of Uganda. Department of Lands and Survey, Entebbe, Uganda.

Azas N, Laurensin N, Delmas F, Di Giorgio, Gasquet M, Laget M, Timon-David P, 2002. Synergistic in vitro antimalarial activity of plant extracts used as traditional herbal remedies in Mali. Parasitology Research 88, 165-171.

Caulfield LE, Richard SA, Black RE, 2004. Undernutrition as an underlaying cause of malaria morbidity and mortality in children less than five years old. Am. J. Trop. Med. hyg. 71(2), 55-63.

Lartey A, 2008. Maternal and child nutrition in Sub-Saharan Africa: challenges and interventions. Proceedings of the Nutrition Society 67, 105-108.

Tabuti JRS, 2008. Herbal medicines used in the treatment of malaria in Budiope county, Uganda. Journal of Ethnopharmacology 116, 33-42.

WHO, (World Health Organisation), 2008. Traditional medicine. Fact sheet Nº134. http://www.who.int/mediacentre/factsheets/fs134/en/ accessed May 26th 2010.

WHO, 2009. World Malaria Report 2009. http://www.who.int/malaria/world_malaria_report_2009/en/index.html , accessed on 5th May 2010.

Wroughton L, 2010. G8 nations to take stock of development needs. June 25 2010. http://www.reuters.com/assets/print?aid=USTRE65O0NJ20100625, accessed 28.06.2010.

Table 1.

Socioeconomic characteristics of informants (n=28)

Characteristics		(%)
Sex	Female	96
	Male	4
Tribe	Munyankole	82
	Nyankole	7
	Omukiga	7
Education	None	36
	Primary 1-4	14
	Primary 5-7	39
	Secundary	11
Religion	Catholic	50
	Protestant	46
Primary job	Farmer	64
	TBA	14
	Business	7
	TH	4
	Nurse assistant	4
Second. job	TBA	39
	TH	18
	Farmer	7
	Housecare taker	4
	Chairperson NGO	4

Table 2.

Malaria symptoms mentioned by respondants

Symptom	(%)
High body temp	75
Shivering	75
Headache	39
Vometing	29
Pale eyes	25
Loss of appetite	25
Weakness, not active	25
Abdominal pain	21
Diarrhea	18
Jaundice/ yellow skin	14
Yellow eyes	11
Goose pimles	11
Fast heart rate	7
Blisters on mouth	7
Thirst	7
Fluctuating temperature	7
Anemic	7
Pale skin/ hands	7
Mental confusion/ hallusinations	7
Backache/ stiff neck	7
Dry lips	4
Yellow placenta	4
Red eyes	4
Constapation	4
Change in colour	4
Urinating yellow urine	4
Cold feet	4
Dizziness	4
Weak but no fever	4
Nausea	4
Vometing yellow	4
Sweating	4
Convultions	4

Table 3.

Factors reported to cause malaria	
Mosquito bites	86
Drinking unboiled water	25
Bad feeding	14
Dirty/ bad environment	11
Worms	7
Related to season	4
AIDS	4
Tubercolosis	4
Cold weather	4
Change of place/location	4
Poor hygiene	4
Sleeping in cold places	4
Cross infection	4
Transis.from bad matress	4
Not drinking enough	4
Over work	4
Grass around homestead	4
Stagnant water	4
Spleen	4
Flactuance	4
Does not know	4

Table 4.
Plants used to treat malaria by members of Nyakayojo TBAs and THs Assosiation (28 informants)

Scientific name	Family	Local name	Specimen number(s)	Part used[a]	No	% Inf.
Vernonia amygdalina	Asteraceae	Omubirizi	111	L	24	86
Aloe sp., wild sp.	Aloeaceae	Rukaka	336	L	19	68
Justicia betonica	Acanthaceae	Quinine	223	L	11	39
Vernonia adoensis	Asteraceae	Nyakajuma	301, 323, 338, 339	L (F)	8	29
Tithonia diversifolia	Asteraceae	Ngaro Itano /Komanyonyoko	376	L (F)	6	21
Bothriocline longipes	Asteraceae	Ekyogayanja	374	L	5	18
Azadirachta indica	Meliaceae	Neem		L	4	14
Clutia abyssinica	Euphorbiaceae	Omubarama	305, 335	L	4	14
Guizotia scabra	Asteraceae	Ekiterankuba	320	L	4	14
Vernonia lasiopus	Asteraceae	Omujuma	306, 340	L	4	14
Aloe sp., cultivated	Aloeaceae	Enkaka		L	3	11
Conyza bonariensis	Asteraceae	Ndasha	22, 330	L	3	11
Crassocephalum manni	Asteraceae	Omusununu/ Entarahonda	315	L	3	11
Cymbopogon citratus	Poaceae	Omuteete		L	3	11
Gynura scandens	Asteraceae	Ekizimya-muriro	402	L	3	11
Microglossa pyrifolia	Asteraceae	Omuvugankande/ Omuhe	207,334, 375,395	R	3	11
Artemisia annua	Asteraceae	Sweet Anne		L	2	7
Bidens granti	Asteraceae	Ehongwa	326	L, F	2	7
Cajanus cajan	Fabaceae	Entondaigwa	377	L	2	7
Carica papaya	Caricaceae	Ipapali / pawpaw		L	2	7
Lantana trifolia	Verbenaceae	Omuhukye	311	L	2	7
Monechma subsessile	Acanthaceae	Erazi	332	L	2	7
Momordica foetida	Cucurbitaceae	Orwihura	322	L	2	7
Musa sp.	Musaceae	Kabalaba/Endere/Banana		L	2	7
Passiflora edulis	Passifloraceae	Akatunda	399	L	2	7
Plectranthus cf. *forskohlii*	Lamiaceae	Ekizera	341	L	2	7
Pluchea ovalis	Asteraceae	Omuneera	328	L	2	7
Pseudarthria hookeri	Fabaceae	Omukongorani	205, 378	L	2	7
Rhynchosia viscosa	Fabaceae	Kashaka-Karyoya/Omutegansi	400	L	2	7
Tetrorchidium didymostemon	Euphorbiaceae	Ekisiranfu	327	L	2	7
Arachis hypogea	Fabaceae	Ebinyobwa		L	1	4
Aristolochia elegans	Aristolochiaceae	Musuja welaba	364	S	1	4
Aspilia africana	Asteraceae	Ekarwe	333	L	1	4
Carissa spinarum	Apocynaceae	Omuyonza	348	R	1	4
Crassocephalum	Asteraceae	Nyakabatura	369	L	1	4

Scientific name	Family	Local name	Specimen number(s)	Part used[a]	No	% Inf.
Heteromorpha trifoliata	Apiaceae	Omume(me)na	329	L	1	4
Hoslundia opposita	Lamiaceae	Esitaimwe	107, 208	F	1	4
Indigofera amerginella	Fabaceae	Omunyazabashumba	224, 318	Rb	1	4
Indigofera arrecta	Fabaceae	Omushoroza	324	Rb	1	4
Macrotyloma axillare	Fabaceae	Akihabukuru	396	L	1	4
Maesa lanceolata	Myrsinaceae	Omuhanga	390	L	1	4
Markhamia lutea	Bignoniaceae	Omushambya	398	L	1	4
Myrica kandtiana	Myricaceae	Omujeeje		L	1	4
Ocimum lamiifolium	Lamiaceae	Omwenyi	389	L	1	4
Pentas longiflora	Rubiaceae	Ishagara	316	L	1	4
Physalis peruviana	Solanacaea	Amantuntunu	310	L	1	4
Plectranthus sp. ?	Lamiaceae	Akayondo-akakye		L	1	4
Senna didymobotrya	Fabaceae	Omugabagaba	307	L	1	4
Sonchus oleraceus	Asteraceae	Entahutara	325	L	1	4
Siegesbeckia orientalis	Asteraceae	Kyaryaho	392	R	1	4
Tetradenia or *Plectranthus*	Lamiaceae	Omuravunga		Rb	1	4
Toddalia asiatica	Rutaceae	Kabakura	391	R	1	4
Trimeria grandifolia ssp. tropica	Salicaceae	Omwatanshare	393	L	1	4
Unknown		Omuhumuza		L	1	4
Unknown		Omukurajo	304	L	1	4
Unknown		Omusha		R	1	4
Unknown		Orujwamate		L	1	4

[a] F, flower; L, leaf, R, root; Rb, root bark; S, seed

Table 5.
Recipes used to treat malaria by members of Nyakoyojo TBAs and THs Association

Nr	Scientific name * (number of recipes for species)	Plant part used [a]	Mode of preparation	Added substances	Mode of administer.	Special uses
1	*Vernonia amygdalina* (29)*	L (11)*	Juice	Cooled, preboiled water	Oral	Deworming
		L (4)	Decoction		Oral	
		DLP (2)	Maceration	Cooled, preboiled water	Oral	
		L	Juice	water	Bath	Reduce temp. Squeeze on patient
		L	Juice	See recipe with *B. longipes*	Bath	
		L	Juice	See recipe with *Aloe*		
		L	Decoction	See recipe with *B. longipes*	Steambath	
		L	Infusion	*J. betonica* and *V. adoensis* lvs	Oral	
		DLP	Decoction		Oral	
		L & R	Decoction		Oral	
		L & R	Decoction	*V. lasiopus, G. scabra* lvs and Omusha roots (little water)	Oral	
		R	Decoction	*Tetradenia* root bark (48)	Oral	
		R	Decoction	*Siegesbeckia orientalis* roots	Oral	
		Rb	Decoction		Oral	
	V. amygdalina Recipe 9	DLP & DR	Decoction	Lvs & flr of *V. adoensis, T. diversifolia, B. grantii,* flr of *G. scabra* and lvs of *Musa sp. Aloe*indigenous and cult. are added fresh	Oral	The six first plant parts are dried in the shade.
2	*Aloe* sp. 'Rukaka' (indigenous) (18)	L (11)	Decoction		Oral	Jaundice
		L (2)	Juice	Cold water	Oral	
		L	Boil	Banana juice	Oral	
		L	Decoction	Lvs of other plant (unknown)	Oral	
		L	Infusion	*Bidens grantii* leaves when in flower	Oral	Yellow eyes
		L	Juice	*V. amygdalina* and *V.lasiopus* lvs	Oral	
		L	Decoction	See recipe 9 with *V. amygdalina* (1)		
3	*Vernonia adoensis* (12)	L (2)	Decoction		Oral	
		L & F	Decoction		Oral	
		L	Infusion		Oral	

Nr	Scientific name * (number of recipes for species)	Plant part used [a]	Mode of preparation	Added substances	Mode of administer.	Special uses
		L	Juice	Water	Oral	
		L	Infusion	*V. amygdalina* and *J. betonica* lvs	Oral	
		L & F	Decoction	*V. amygdalina* and *J. betonica* lvs	Oral	
3	*Vernonia adoensis* cont.	L & F	Decoction	See recipe 9 with V. amygdalina (1)	Oral	
		L	Decoction	See recipe with *C. abyssinica* (10)	Oral	
		L	Decoction	*Guizotia scabra* lvs,	Oral	
		L	Decoction	See recipe with *B. longipes* (8)	Steambath	
		R	Decoction		Oral	Spleomegaly
4	*Justicia betonica* (10)	L (4)	Decoction		Oral	Flatulence,
		L	Decoction	Omuhumusa	Oral	cough,
		L	Decoction	*Bothriocline longipes* lvs	Oral	
		L	Infusion		Oral	Worms
		L	Infusion	*V. amygdalina* and *V. adoensis* lvs	Oral	
		L	Juice	Cooled, preboiled water	Oral	
		DLP	Decoction		Oral	
5	*Tithonia diversifolia* (5)	L & F(2)	Decoction		Oral	
		L	Decoction		Oral	
		L & F	Decoction	See recipe 9 with *V. amygdalina* (1)	Oral	
6	*Guizotia scabra* (5)	L	Infusion		Oral	
		L	Decoction	See recipe with *V. amygdalina* (1)	Oral	
		L	Decoction	*V. adoensis* lvs	Oral	
		L	Decoction	See recipe with *B. longipes* (8)	Steam bath	
		L	Decoction	See recipe 9 with *V. amygdalina* (1)	Oral	
7	*Azadirachta indica* (5)	L (3)	Decoction		Oral	
		L	Decoction	See recipe with *Aloe,* (Enkaka, 9)	Oral	
		L	Infusion		Oral	
8	*Bothliocline longipes* (5)	L	Decoction	See recipe with *Musa* sp. (24)	Steam bath	
		L	Decoction	*Aspilia africana* lvs	Oral	
		L	Decoction	See recipe with *J. betonica* (4)	Bath	
		L	Juice	*V. amygdalina,* Ekizimya-muriro, Ekiziranfu lvs, cold water	Bath, squeeze on patient	
		L	Decoction	*V. amygdalina, V. adoensis, G. scabra,*	Steambath, then	

Nr	Scientific name * (number of recepies for species)	Plant part used [a]	Mode of preparation	Added substances	Mode of admini-	Special uses
9	*Aloe* sp. 'Enkaka' (cultivated) (4)	L	Juice		Oral	
		L	Decoction		Oral	
		L	Decoction	*Azadirachta indica*	Oral	
		L	Decoction	See recipe 9 with *V. amygdalina* (1)	Oral	
10	*Clutia abbysinica* (4)	L	Infusion	See recipe with *P. peruviana* (44)	Oral	Splenomegaly
		L	Decoction	*V. adoensis* and *T. grandifolia* lvs, lemon peel	Oral	
		L	Juice	Cooled, preboiled water	Oral	
		L	Decoction	*Heteromorpha trifoliata* lvs	Oral	
11	*Vernonia lasiopus* (4)	L	Decoction		Oral	
		L	Juice		Oral	
		L	Decoction	See recipe with *V. amygdalina* (1)	Oral	
		L	Juice	See recipe with *Aloe* (2)	Oral	
12	*Gynura scandens* (4)	L (2)	Juice		Bath and oral	Reduce temp.
		L	Juice	See recipe with *B. longipes* (8)	Bath	
		L	Decoction	See recipe with *B. longipes* (8)	Bath	
13	*Cajanus cajan* (3)	L	Maceration	Omutegansi, Kashaka-karyoya	Oral	Splenogalmy
		L	Maceration	2. Milk, Omutegansi, Kashaka-karyoya	Oral	Diarrhoea
		L	Maceration		Oral	
14	*Conyza bonariensis* (3)	L (2)	Decoction		Oral	Overdose: diaorrhea
		L	Juice	Cooled, preboiled water		
15	*Crassocephalum manni* (3)	L	Juice	See recipe with *M. foetida*	Oral	
		L (2)	Infusion		Oral	
16	*Cymbopogon citratus* (3)	L	Decoction		Oral	
		L	Decoction	See recipe with *H. oposita* (38)	Oral	
		L	Decoction	*C. papaya* lvs	Oral	Reduce temp.
17	*Momordica foetida* (3)	L	Decoction	Banana and *B. longipes* lvs.	Steam bath	
		L	Decoction	See recipe with *Musa* sp. (24)	Steam bath	
		L	Juice	*L. trifolia, C. manni* lvs and water	Oral	
18	*Pluchea ovalis* (3)	L	Decoction		Oral	

Nr	Scientific name * (number of recepies for species)	Plant part used [a]	Mode of preparation	Added substances	Mode of admini-	Special uses
		L	Maceration	Rock salt	Oral	Incr. apatite, diarrhoea
		DLP	Decoction		Oral	Constipation
19	*Artemisia annua* (2)	L	Decoction		Oral	Profylact.: drink once
20	*Carica papaya* (2)	L	Decoction	Lemon gras	Oral	
		R	Decoction			
21	*Lantana trifolia* (2)	L	Juice	Water	Oral	
		L	Juice	See recipe with *M. foetida* (17)	Oral	
22	*Monechma subsessile* (2)	L	Decoction	See recipe with *H. oposita* (38)		
		L	Infusion	See recipe with *P. peruviana* (44)	Oral	Splenomegaly
23	*Microglossa pyrifolia* (2)	L	Decoction		Oral	For constipation
		L	Decoction	See recipe with J. betonica (4)	Bath	
24	*Musa sp.* (2)	L	Decoction	*Bothriocline longipes* and *Momordica foetida* lvs	Steam bath	
		L	Decoction	See recipe 9 with *V. amygdalina* (1)		
25	*Passiflora edulis* (2)	L (2)	Juice	Cooled, preboiled water		
26	*Plectranthus* cf. *forskohlii* (2)	L (2)	Decoction		Oral	
27	*Tetrorchidium didymostemon* (2)	L	Juice	Cold water	Bath	
		L	Decoction	See recipe with *B. longipes* (8)	Bath	
28	*Bidens grantii* (1)	L & F	Decoction	See recipe 9 with *V. amygdalina* (1)		
29	*Pseudarthria hookeri* (2)	L	Decoction	See recipe with *H. opposita* (38)	Oral	
			Decoction		Oral	Reduce temp.
30	*Senna didymobotrya* (2)	L	Decoction		Oral	Children with
		or DLP	Add in food		Oral	splenomegaly
31	*Rhynchosa viscosa* (2)	-	Maceration	See recipe with *C. cajan* (13)	Oral	
32	*Arachis hypogea* (1)	L	Juice	Cooled, preboiled water	Oral	
33	*Aristolochia elegans* (1)	S	Maceration		Oral	
34	*Aspilia africana* (1)	L	Decoction	*Bothriocline longipes*	Oral	
35	*Carissa spinarum* (1)	R	Decoction	*T. asiatica*	Oral	
36	*Crassocephalum* sp. (1)	L	Infusion		Oral	
37	*Heteromorpha trifoliata* (1)	L	Decoction	*Clutia abyssinica* lvs	Oral	

Nr	Scientific name * (number of recepies for species)	Plant part used [a]	Mode of preparation	Added substances	Mode of admini-	Special uses
38	*Hoslundia opposita* (1)	F	Decoction	lvs of *C. citratus, P. subsessile,*	Oral	Abdominal
39	*Indigofera amerginella*	Rb	Infusion		Oral	
40	*Indigofera arrecta* (1)	Rb	Decoction		Oral	Reduce temp.
41	*Maesa lanceolata* (1)	L	Maceration		Bath	Yellow fever
42	*Markhamia lutea* (1)	L		Akayondo-akakye	Oral	
43	*Microglossa pyrifolia* (1)	R	Decoction		Oral	
44	*Ocimum lamiifolium* (1)	L	Infusion	See recipe with *P. peruviana* (44)	Oral	Splenomegaly
45	*Physalis peruviana* (1)	L	Infusion	*M. subsessil, C. abbyccinica*	Oral	Splenomegaly
46	*Plectranthus* sp. (1)	L	Juice	*Markhamia lutea* lvs, water	Oral	Stop vometing
47	*Siegesbeckia orientalis* (1)	R	Decoction	*V. amygdalina*	Oral	
48	*Sonchus oleraceus* (1)	L	Infusion		Oral	
49	*Tetradenia* or *Plectrunthus* (1)	Rb	Decoction	See recipe with *V. amygdalina* (1)	Oral	
50	*Toddalia asiatica* (1)	R	Decoction	*C. spinarum* roots	Oral	
51	*Trimeria grandifolia ssp. tropica* (1)	L	Decoction	See recipe with *C. abyssinica* (10)	Oral	
52	*Macrotyloma axillare* (1)	L		See recipe with *H. opposita* (38)	Oral	
53	*Pentas longiflora* (1)	L	Boil juice	Water	Oral	Constipation
54	Omuhumuza (1)	L	Decoction	See recipe with J. betonica (4)	Oral	
55	Orujwamate (1)	L	Juice	Water	Oral	
56	Omukura(i)jo (1)	L	Decoction		Oral	Toxic, little at a time
57	Omusha (1)	L	Decoction	See recipe with *V. amygdalina* (1)	Oral	

* Consensus, as number of citations are written in paranthesis for each species and plant part used
When several plants are used together the whole resipe is given by the plant that is mentioned first in the recipe. For the other plants it is pointed to the recipe.

[a] DLP, dry leaf powder; F, flowers; L, leaves; R, roots; Rb, root bark; S, seeds; when only used once there is no number behind

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