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Ethnobotanical use, threat status and optimalenvironmental germination conditions for conservation of aloe species in Tanzania

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ETHNOBOTANICAL USE, THREAT STATUS AND OPTIMALENVIRONMENTAL GERMINATION CONDITIONS FOR CONSERVATION OF *ALOE* SPECIES IN TANZANIA

Siri Abihudi

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

The genus *Aloe* is known for its use in healthcare and cosmetics. Many *Aloe* species are globally threatened due to over-harvesting for trade and habitat destruction. In Tanzania, over-exploitation threats some *Aloe* species with extinction, and yet little has been documented on the abundance, bio-cultural uses, threats, seed biology and appropriate conservation measures. Semi-structured questionnaires were used to obtain ethnobotanical information and perception of *Aloe* species' threat factors from 236 respondents in Kilimanjaro, Tanga, and Mara and Katavi-Rukwa regions. Geospatial Conservation Assessment software (GeoCAT) was used to assess the Area of Occupancy and Extent of Occurrence of *Aloe* species. The temperature, light, scarification, KNO_3 and salinity among the Critically Endangered and the Least Concern *Aloe* species were tested to understand optimal environmental conditions for their optimal growth. For the Critically Endangered *A. boscawenii*, an ideal concentration for sterilization and rooting hormone were determined to promote tissue cultivations. A total of 23 *Aloe* species were identified, with *A. secundiflora* being the most widely used species. Malaria and general stomachache in humans, and Newcastle disease in chickens were frequently treated with *Aloe* species. Among the 22 *Aloe* species re-assessed, two were categorized as Critically Endangered, ten as Endangered, five as Vulnerable, one as Nearly Threatened and five as Least Concern. *Aloe boscawenii* was re-discovered after not having been seen for more than six decades. The conservation status of the *Aloe* species endemic to Tanzania and previously categorized as Critically Endangered were upgradeed. All the tested *Aloe* species germinated best at moderate temperatures (25°C and 30°C) and low KNO_3 levels (0.01 mg/L). The Critically Endangered *A. boscawenii* was successfully regenerated through tissue culture at 6% of NaOCl and rooted at NAA: IBA (2:1) concentration. The genus *Aloe* is widely used across Tanzania, as such 77% (N = 22) of *Aloe* species assessed in this study are threatened, mainly due to human activities. Hence, there is a need to formulate laws and policies to protect *Aloe* natural habitats. Moreover, the *Aloe* seeds exhibited species-specific responses to various environmental conditions (except for *A. pembana*). Furthermore, tissue culture is an ideal conservation tool for the threatened species as it outperforms the traditional methods.

DECLARATION

I, Siri Abihudi, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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CERTIFICATION

The undersigned certify that they have read the dissertation titled *"Ethnobotanical use, threat status and optimal environmental germination conditions for conservation of Aloe species in Tanzania"* and recommend for acceptance in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

I am dedicating this dissertation to my beloved the late father; Abihudi Abymike Mlungwana, my late sister; Elizabeth Abymike, my beloved mum, my niece and nephew Furaha and Faraja, my beloved brothers (Mr. Solomon, Mr. Katanga, Mr. Amani, and Mr. Meshack), My uncles (Mr. Simeon and Mr. Mbonea) and My Omulabe (Bukirwa Mayanja Daphne).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent sign
±	Plus–minus sign
°C	Centigrade
AAU	Aarhus University, Denmark
ANOVA	Analysis of variance
AOO	Area of Occupancy
APG	Angiosperm Phylogeny Group
BA	Benzyl adenine
BC	Before Christ
$C_2H_3O_2$	Acetate Ion
Ca	Calcium
CEC	Cation exchange capacity
CITES	Convention on International Trade in Endangered species of Fauna and Flora
cm	Centimeter
CR	Critically Endangered
D&N	Day & Night
DES	Desert Botanical Garden, United States
DSM	University of Dar es Salaam, Tanzania
DTPA	diethylene triamine pentaacetic acid
DW	Distilled Water
E	Margin of error
EAM	Eastern Arc Mountains
EC	Electrical conductivity
EDTA	Edetate disodium
EN	Endangered
EOO	Extent of Occurrence
Fe	Iron
FGP	Final Germination Percentage
g	Gram
GBIF	Global Biodiversity Information Facility
GeoCAT	Geospatial Conservation Assessment Tool
GIS	Geographical Information System
h	Hour
$Hg(NO_3)_2$	Mercury (II) nitrate
IBA	Indole-3-butyric acid
IBM	International Business Machines
ITM	Institute of Traditional Medicine
IUCN	The International Union for Conservation of Nature
K	Royal Botanic Gardens Kew, United Kingdom
K	Potassium
km	Kilometer
KNO3	Potassium nitrate

l	Litre
LC	Least Concern
m.a.s.l	Meters Above Sea Level
mg	Milligram
Mg	Magnesium
Min	Minutes
ml	Milliliter
Mn	Manganese
MO	Missouri Botanical Garden, United States
MS	Murashige and Skoog
MUHAS	Muhimbili University of Health and Allied Sciences
n	Sample size
NAA	α-Naphthalene acetic acid
NaOCl	Sodium hypochlorite
NE	Not Evaluated
NH_4	Ammonium acetate
NHT	National Herbarium of Tanzania, Tanzania
NM-AIST	Nelson Mandela African Institution of Science and Technology
NT	Near Threatened
OC	Organic carbon
P	Phosphorus
PVP	Polyvinylpyrrolidone
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
SSC	Species Survival Commission
TAWIRI	Tanzania Wildlife Research Institute
N	Nitrogen
TPRI	Tropical Pesticides Research Institute
VU	Vulnerable
w/v	Weight/Volume
α	Significance level
σ	Standard derivation

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

The genus *Aloe*, also written Aloë comprises about ± 630 species of succulent flowering plants (Klopper *et al.*, 2013). *Aloe vera* (L.) Burm.f., or true Aloe, is the most commonly known species described in 1753 by Carl Linnaeus as *Aloe perfoliate* L. var. vera L. Currently, *Aloe* falls under the *Asphodelaceae* family following the Angiosperm Phylogeny Group (APG IV) classification in 2016 (Chase *et al.*, 2016) from the older APG III classification which placed it in the a *Xanthorrhoeaceae* family (Angiosperm Phylogeny Group, 2009; Carter *et al.*, 2011). *Aloe* is a genus of evergreen perennial herbs, shrubs or small trees native to tropical and southern Africa, Madagascar and the Arabian Peninsula. It grows in the wild in tropical, semi-tropical and arid climates across a wide range of elevations and habitat types, including grassland, rocky areas, coastal sites and cliff faces worldwide (Arena *et al.*, 2015; Carter, 1994; Carter *et al.*, 2011; Newton, 2004). Sub-Saharan Africa, including the island of Madagascar, accounts for over 90% of the 630 species of the genus *Aloe*, with a concentration in Southern Africa (over 260 taxa), in Eastern Africa (over 180 taxa) and on the island of Madagascar (over 77 taxa) (Grace *et al.*, 2015; Newton, 2004; Fig. 1). A taxon is a collection of one or more populations of an organism or organisms that taxonomists regard as forming a unit.

Tanzania is a biodiversity-rich ecosystem (Newmark, 2002; Tabor *et al.*, 2010), in which 52 taxa represent the genus *Aloe*, where 24 are endemic to Tanzanian mainland, and two are found in Zanzibar (Carter, 1994; Reynolds, 2004; Wabuyele, 2006). High biodiversity regions for *Aloe* species in Tanzania include Katavi-Rukwa (8 species), Kilimanjaro (8 species), and Tanga (7 species) regions (Carter, 1994). Further, the study by Wabuyele *et al.* (2006) in few villages in Tanga, Arusha and Mbeya discovered several new species, namely: *Aloe cataractarum* T. A. McCoy and Lavranos, *Aloe kwasimbana* T. A. McCoy and Lavranos, *Aloe ruvuensis* T. A. McCoy and Lavranos and *Aloe latens* T. A. McCoy and Lavranos. According to The International Union for Conservation of Nature (IUCN), it is shocking to note that about quarter (23%) of *Aloe* species are threatened (IUCN Red List website), while the population trend of some species is unknown (Wabuyele & Kyalo, 2008). Human activities such as deliberately set fires, the extension of agricultural land, human settlements and over-exploitation, are among the potential causes for the extinction of *Aloe* species (Convention on International Trade in Endangered species of Fauna and Flora [CITES], 2003). These factors have caused a shift in species range, extinction of other medicinal plants (Foden *et al.,* 2007) and loss of genetic diversity (Thuiller *et al.,* 2008). The Convention on International Trade in Endangered species of Fauna and Flora (CITES) ensures that international trade in both flora and fauna does not threaten their survival. In 1979 Tanzania signed the Convention on International Trade's regulations in Endangered Species of Fauna and Flora, i.e. CITES (Majamba, 2000).

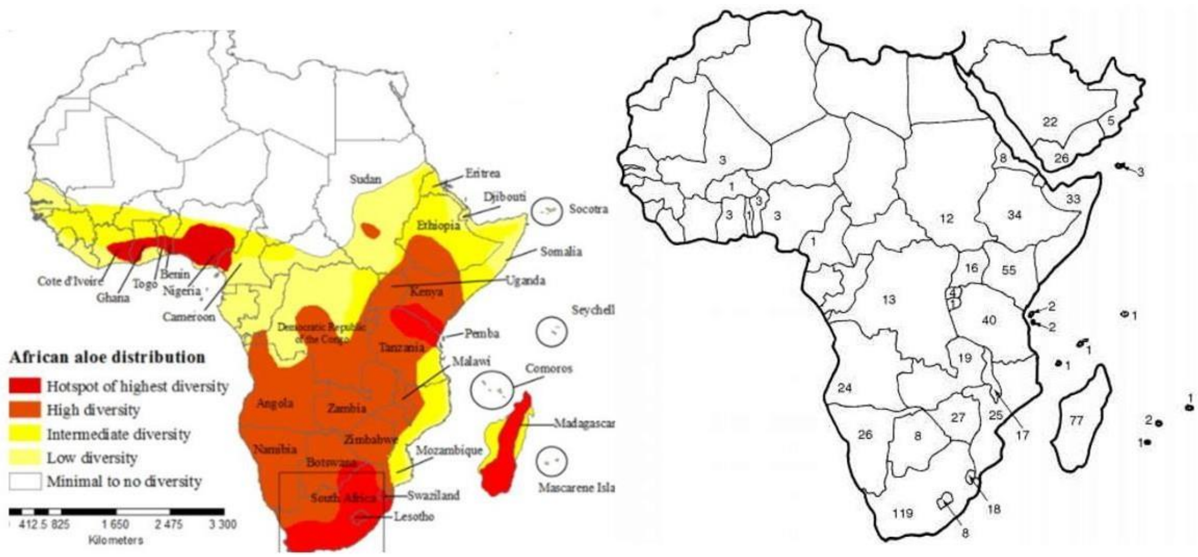

Figure 1: A map showing the distribution of *Aloe* species on the African continent with the left-hand side map showing the diversity of *Aloe* species (Cousins & Witkowski, 2012), and the right-hand side graph showing the number of *Aloe* species found per country in the year 2004 (Reynolds, 2004)

Although there are grass *Aloe* (Bjorå *et al.,* 2015), these green succulent plants can mostly be easily distinguished from other species by their triangular, fleshy, serrated leaves (Surjushe *et al.,* 2008). Their succulent nature provides high physiological and structural adaptation to the arid environmental regions (Grace *et al.,* 2015; Newton, 2004). The juicy nature of the leaves allows high storage of water to be held in the mucilage. Most of the species in the genus give yellow exudate when the leaf is cut, produced in the aloitic cells. The yellowish exudate always contains chemical compounds that are a potential source of medicine to various communities, although some turn to be poisonous (Reynolds, 2004).

The use of *Aloe* as medication dates back to the Sumerians (BC 2100) and has one of the most extended histories to be used in traditional medicine worldwide (Basmatker *et al.,* 2011; Haller, 1990). To date, the use of *Aloe* species is widespread in traditional and pharmaceutical medicine due to their potential for its anti-inflammatory, anti-arthritic, anti-bacterial and hypoglycemic effects (Manvitha & Bidya, 2014; Pandey & Mishra, 2010; Shahzad & Ahmed, 2013; Yebpella *et al.,* 2011).

Despite the genus being acknowledged for its efficacy in traditional healing and commercial products worldwide, few species (10) feature in the international trade, with the *Aloe vera* leading (Grace *et al.*, 2015). Other famous *Aloe* species in the global market include *Aloe vera* and *Aloe aborescens* Mill (Smith *et al.*, 2008; Radha & Laxmipriya, 2015; Verma, 2011), *Aloe ferox* Mill (Dagne *et al.*, 2000; Grace, 2011; Radha & Laxmipriya, 2015; Sachedina & Bodeker, 1999) and *Aloe secundiflora* Engl. (Grace, 2011). Due to the high demand worldwide, threats by illegal collection and trade of most *Aloe* species including *A. ferox* (Knapp, 2006), *A. aborescens* (Smith *et al.*, 2008) and *A. secundiflora* (CITES, 2003), conservation of *Aloe* is of vital importance. The CITES regulates trade in most Aloes, except for *Aloe vera* which can be cultivated for commercial purposes.

Different techniques including RAMAS Red List (Version 2.0) have been used in the assessment of species by scientists (Pfab & Victor, 2002), Geographical Information System (GIS) based on modelling tools such as BIOMAPPER (Lobo & Chefaoui, 2005) and ArcGIS (Kutnar & Hladnik, 2016) and IDRISI for Windows are used to calculate the Area of Occupancy (AOO) that is the area within its 'Extent of Occurrence (EOO) which is occupied by a taxon, excluding cases of vagrancy. The mentioned techniques require computation skills and they can take longer to accomplish. Geospatial Conservation Assessment software (GeoCAT) is an online assessment program that utilizes IUCN Red List criterion B, restricted geographic range, to categorize the species. Both the field generated data and the online data such as GBIF, Flickr, and Scratchpads can be used. In comparison to other assessment methods, GEOCAT provides information on specimens or their occurrence on a global scale (Bachman *et al.*, 2011). Many Aloes are regarded as threatened with possible extinction in the future (Bachman *et al.*, 2020). Various threats exist, and they can be placed into three main categories: Over-collection of plants for cultivation, destruction of plants in harvesting leaf exudates and destruction of natural habitats (Reynolds, 2004b).

The threat to most of *Aloe* species is often associated with low seed establishment (Phama *et al.*, 2014), retarded growth (Pfab & Scholes, 2004), low competitive ability (Markham, 2014), seed quality (Arena *et al.*, 2015), absence of pollinator species (Botes *et al.*, 2008; Cousins *et al.*, 2013) and prolonged seed dormancy (Cousins *et al.*, 2014). Therefore, knowledge of *Aloe* species' germination behaviour is essential in mitigating the negative effect of changing environmental conditions as the result of global warming and prospects for conservation. The increase in temperature and drought as the result of global warming results in increased mortality and decline of populations, a case in point being that of the four-decade modelling of *Aloe dichotoma* in the Namib Desert in Namibia (Foden *et al.*, 2007). Moreover, Seeds play a major role in species persistence, namely reproduction, dispersion, development to new habitats and seasonally environmental survival of germplasm (Vázquez-Yanes & Orozco-Segovia, 1993). These examples justify the need to study the seed ecology and germination behaviour of *Aloe* species. The findings will help in determining favourable conditions for the germination survival and conservation of *Aloe* species.

Aloe boscawenii is the rarest and is among the five Critically Endangered (CR) *Aloe* species that grow along the coast of Tanzania mainland (Carter, 1994; Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b). The species is known to occur in two locations in a tiny geographical range. The latest attempt to locate it by Eastern Arc Mountains and Coastal Forests CEPF Plant Assessment Project Participants (2009b) was unsuccessful. The factors for the rapid decline for its population are not known, and there is no record for its cultivation. Hence the current status calls for immediate attention to develop techniques to increase its abundance in the wild, thus preventing the species from extinction. Tissue culture, a new propagation technology has been investigated to replace the traditional seed germination, which suffers from seed dormancy and the longer germination times (Wochok, 1981).

1.2 Statement of Problem

Aloe species have been used for medicinal purposes and cosmetics for centuries (Bland *et al.,* 2016; Grace, 2011). Use of *Aloe* species by local people in Tanzania has become common because of their accessibility and present almost no or little side effects when used (Pili, 2007). However, the documentation on use and bio-cultural value of the *Aloe* species in Tanzania is limited. The study by Amir *et al.* (2019) is restricted to indigenous knowledge from only a few areas, namely Mbozi, Kigoma, Mkweni and Morogoro. There is a need to explore the ethnobotanical use of Aloes in other parts of Tanzania. For example, there is a diversity of *Aloe* around the Serengeti ecosystem, which has never been explored (Manoko, 2015), and ethnobotanical survey of this study will focus on ecosystem areas with a similar diversity of *Aloe*.

Almost a third of *Aloe* species have limited distribution in East Africa and are locally threatened (Carter, 1994; Wabuyele *et al.*, 2006). Regardless of their endemism nature, only 54% of *Aloe* species have been assessed and categorized according to the IUCN Red List (CITES, 2003). There is a need for Not Evaluated (NE) *Aloe* species to be categorized, but previously evaluated species have to be updated (CITES, 2003). Some *Aloe* species are Critically Endangered (CR) due to human activities, including over-harvesting for medicinal uses and land use changes (CITES, 2003; Wabuyele *et al.*, 2006). This has put five Tanzanian *Aloe* species to a CR state reported by IUCN

Red List (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009d, 2009b, 2009e, 2009f, 2009h). Such an assessment of *Aloe* species will also highlight the conservation of threatened species in Tanzania.

The Least Concern (LC) *Aloe* species such as *A. lateritia* and *A. secundiflora* are widely distributed in Tanzania and Kenya (Weber, 2013; Weber & Demissew, 2013b). In contrast, the CR *Aloe* species such as *A. boscawenii* and *A. pembana* are endemic to Tanzania with restricted distribution (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b, 2009h). Moreover, there is no established link between the germination behaviour of the LC (*A. lateritia, A. volkensii* and *A. secundiflora*) and CR (*A. boscawenii* and *A. pembana*) *Aloe* species in Tanzania. Therefore, there is a need for experimental studies on seed germination for the CR and LC *Aloe* species. This helps the scientist understand whether the rarity and endemism or broad distribution of *Aloe* species is linked to seed germination.

Aloe boscawenii is one of the five CR *Aloe* species and has not been sighted for more than half a century at Boma ward, Tanga district (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b). The species is estimated to have a minimal AOO if it still exists. Furthermore, there is no record of the cultivated species, although it is supposed to be found in unnamed forest reserves (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b), probably Kilulu forest reserve (Status Reports for 7 Coastal Forests in Tanga Region, 1989).

The traditional propagation of plant species is challenged with seed dormancy, which results in one individual and a long period of restoration (Wochok, 1981). The side-shoot of *Aloe* is also very slow, with at least 3 to 4 per year, which cannot sustain its conservation (Shekhawat *et al.,* 2013). The propagation method such as in-vitro culture will lead to multiple individuals of *A. boscawenii* in a short period of time. Regardless of numerous protocols developed for in-vitro culture for *Aloes* elsewhere (Bairu *et al.*, 2009; Gupta, 2014; Hashem & Kaviani, 2010), there is none for Tanzanian species. Therefore, the development of in-vitro culture for the threatened species such as *A. boscawenii* in Tanzania should be regarded as a high priority.

1.3 Rationale of the Study

This study aims to document the ethnobotanical knowledge of *Aloe* species, considering that little has been done in Tanzania (Amir *et al.*, 2019). Similar studies in Kenya and Ethiopia which have resulted into expanded list of *Aloe* species and new records (Dessalegn, 2006; Wabuyele *et al.*,

2006) established the taxonomic status of several species. A perusal of the earlier author (Wabuyele, 2006) shows new records to the Tanzania list indicating that such a study was needed.

Most of the IUCN Red List assessed *Aloe* species (54%) need to be updated, and those not assessed, need to be assessed according to the IUCN categories. This will support the IUCN Red List effort to ensure all the species are assessed. The threat level for each species in the high biodiversity area for *Aloe* species will be established through the assessment, which will help set the conservation priority for the regions and highly-threatened *Aloe* species.

Conservation of species using traditional propagation method face some challenges including seed dormancy and the amount time that it takes. On the other hand, the application of tissue culture takes a shorter time with a high multiplication rate. Much has been published on the implementation of the latter in the commercial trade (Bairu *et al.,* 2009; Gupta, 2014; Natali *et al.,* 1990) but little in the conservation of threatened species (Abrie & van Staden, 2001; Liao *et al.,* 2004; Rowntree *et al.*, 2011). Tissue culture technology will permit the conservation of the CR *A. boscawenii*. This will help in the restoration of the species in the sites where it was previously found.

1.4 Research Objectives

1.4.1 Main Objective

This study's main goal was to document the ethnobotanical uses of *Aloe* species, their threat status, germination behavior and guaranteeing the future availability of threatened species with the emphasis on the CR *A. boscawenii*.

1.4.2 Specific Objectives

(i) To assess the ethnobotanical knowledge and conservation strategies conducted by the local communities regarding the *Aloe* species in the *Aloe* high diversity regions of Tanzania.

(ii) To re-assess the conservation status of 23 *Aloe* species in Tanzania using IUCN Red List categories.

(iii) To determine whether the CR *Aloe* species' seed germination shows narrow tolerance to environmental parameters compared to Least Concern (LC) *Aloe* species in Tanzania.

(iv) To evaluate the application of tissue culture and propagation trials in the conservation of *Aloe boscawenii* both in-situ and ex-situ.

1.5 Research Questions

(i) What is the status of ethnobotanical knowledge about *Aloe* species by local communities living in selected sites in Tanzania?

(ii) What influence can both the GeoCAT programme and respondents' perceptions have in setting conservation priorities for *Aloe* species in Tanzania?

(iii) How has the conservation status of endemic *Aloe* species changed in comparison to the previous assessments in Tanzania?

(iv) Which part of the *A. boscawenii* plant will perform better in tissue culture?

(v) Are there optimal environmental conditions that are general shared by the five selected *Aloe* species?

(vi) How does the germination of CR and LC *Aloe* species strive in varying environmental conditions in Tanzania?

(vii) How can the CR species *A. boscawenii* be conserved in Tanzania (through cuttings, traditional germination or tissue culture)?

1.6 Research Hypotheses

(i) Ethnobotanical knowledge (number of uses of *Aloe* species named by each respondent) is higher in older people and more common in women than men.

(ii) The respondent perception of *Aloe* species abundance does not influence the number of uses of *Aloe* species.

(iii) The LC *Aloe* species react differently to environmental parameters in comparison to CR *Aloe* species.

(iv) The α-Naphthalene acetic acid (NAA) and Indole-3-butyric acid (IBA) will perform better when used individually rather than in combination in rooting of *A. boscawenii*.

1.7 Significance of the Study

Various stakeholders will benefit from this study as follows:

(i) The IUCN Red List will update the conservation status of the *Aloe* species that have been discussed in this work. Some *Aloe* species such as *A. dorotheae* A. Berger will change in their status as the result of this study. This will help in conserving threatened *Aloe* species in Tanzania and give more attention to other *Aloe* species at the international level.

(ii) Seminars and presentations were given on the threat level and ways to be conserved at various non-scientific platforms such as the yearly NANENANE event and scientific conferences such as Tanzania Wildlife Research Institute (TAWIRI) Muhimbili University of Health and Allied Sciences (MUHAS), Nelson Mandela African Institution of Science and Technology (NM-AIST) and Agroecology Conferences. Such presentations will be conducted whenever the chance arises.

(iii) The propagules of the CR *A. boscawenii* were distributed for conservation purposes at the MUHAS farm in Lushoto district, Tropical Pesticides Research Institute (TPRI) and NM-AIST botanical garden in Arusha. Furthermore, propagules and knowledge on how to best propagate the plant by locals were distributed to three primary schools and two secondary schools in the Mkinga district. This micropropagation technique can also be applied to the conservation of other threatened species such as *Hypoxis* species.

(iv) Different *Aloe* species propagated during germination experiment were distributed to various institutions such as TPRI, MUHAS and NM-AIST botanical garden. The knowledge on germination of CR and LC *Aloe* species reported in this study were communicated to the local communities during NANENANE event and soapbox. Locals can apply such knowledge in propagation of *Aloe* species for trade which will reduce pressure in the wild. Furthermore, different products will be developed and marketed locally and internationally after successfully propagating of *Aloe* species on a large scale.

1.8 Delineation of the Study

Ethnobotanical knowledge and perceptions about the potential extinction of *Aloe* species were gathered from five regions: Kilimajaro, Tanga, Mara, Katavi, and Rukwa. To complete the genus *Aloe* in Tanzania, more research is needed to capture ethnobotanical knowledge from other regions. Among the 52 *Aloe* species, 22 species were assessed for their conservation status and threat level based on their EOO, AOO, and locality. The remaining 29 taxa in Tanzania must be updated to complete the genus *Aloe*. Different tools, such as RAMAS Red List, IDRISI for Windows, BIOMAPPER, and Geographical Information System (GIS) based on modeling tools, can be used in the assessment depending on the nature of the plants and the availability of data.

The majority of the *Aloe* species were collected during their flowering season and only a few (five) *Aloe* species with seeds were used in the germination experiment. Future research should expand the *Aloe* sample for seed germination to determine whether the rarity of CR or the availability of LC *Aloe* species corresponds to their IUCN Red List status. Additionally, the germination experiment was carried out in a controlled environment. As a result, it would have been more practical to carry out the experiment in the exact location where the species is found. Furthermore, the length and duration of the experiments should have been increased to cover the entire growth stages of the tested *Aloe* species to maturity in their natural field conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conceptual Framework

Figure 2 presents the conceptual framework about what study was all about, what it entails and what were covered during the review of literatures. Therefore, the mains aspects of the current study are summarized within this conceptual framework.

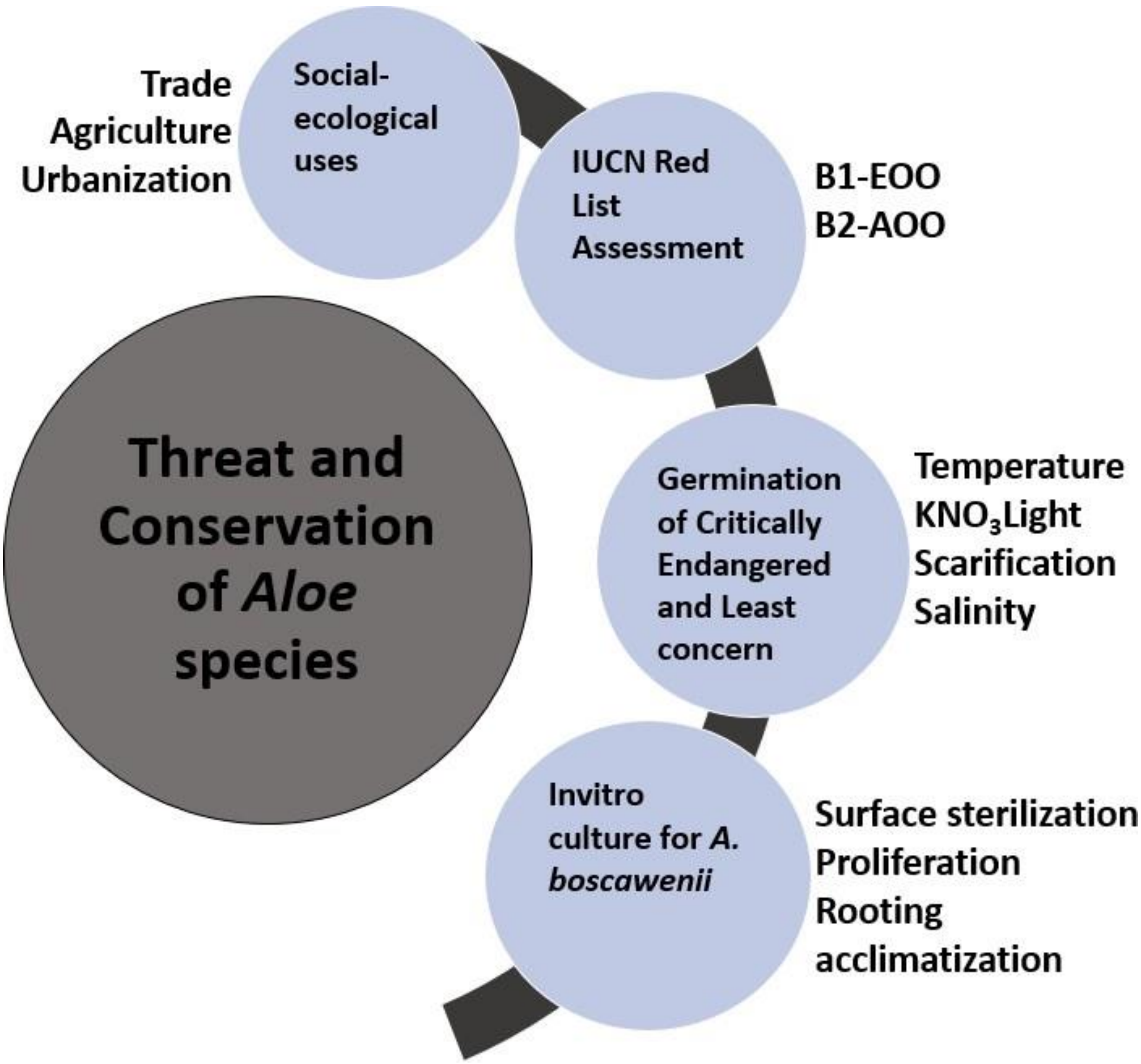

Figure 2: Conceptual framework for the "Socio-Ecological, Red List Re-Assessment, and Conservation of *Aloe* species (*Asphodelaceae*) in Tanzania

2.2 Uses and Biocultural Values of *Aloe* Species

The genus *Aloe* is deduced to have originated in southern Africa 19 million years ago and later (16 million years ago) spread northeastward to the Ethiopian-Somalian and Zambezian region. It later spread towards Madagascar, the Saharan-Sudanian region, West Africa, and the Arabian Peninsula (for a map of Africa see Fig. 1) (Cousins & Witkowski, 2012; Reynolds, 2004b). Although the genus originated in southern Africa, its species endemism is highly experienced in Madagascar and the Islands in the Indian ocean due to high speciation during Gondwana separation (Grace *et al.*, 2015; Newton, 2004). Many countries in mainland Africa have some endemic *Aloe* species, especially large countries such as South Africa and Tanzania compared to other small countries such as Rwanda and Burundi. Moreover, some mainland African species are widely distributed with high EOO, e.g, *Aloe buettneri* A. Berger, which range from Mali to Zambia and *Aloe myriacantha* (Haw.) Schult. and Schult.f. which ranges from East Africa to South Africa (Reynolds, 2004b).

Aloe is a genus containing species, subspecies and varieties (Carter *et al.*, 2011; Dold & Cocks, 2002). The genus occupies a wide range of habitats, from forests to exposed rock surfaces and cliff faces. The paleotropical genus *Aloe* holds a considerable altitude range, from sea level (e.g., *Aloe boscawenii* Christian, *Aloe kilifiensis* Christian) up to about 3500 m.a.s.l (e.g. *Aloe ankoberensis* M. G. Gilbert and Sebsebe, *Aloe steudneri* Schweinf.ex Penz) (Reynolds, 2004).

Human knowledge of the genus *Aloe* dates back to the Sumerians (BC 2100) and is one of the oldest recorded plants known to humankind (Crosswhite & Crosswhite, 1984). The antiquity of this knowlegde was further confirmed by the Egyptian papyri, more than three and a half thousand years ago (Baumann & William, 1960). Alexander the Great and Christopher Columbus used it to treat soldiers wounds (Tanwar *et al*., 2011). Cleopatra and Nefertiti, Egypt's queens, used it for beauty regime. Moreover, it has been used as cholagogue, detergents, vermifuge, antiseptic, insecticide, and used in making wine (Haller, 1990). Having been used for many centuries, *A. vera* has amassed numerous nicknames ranging from “shining bitter substance” to ‘the plant of immortality (Dagne *et al.*, 2005; Tanwar *et al*., 2011).

The immense bio-cultural value of the leaf-succulent genus *Aloe* in Africa is evident in the extensively documented traditions of use (Grace *et al.*, 2009). Many African societies use *Aloe* species in their immediate surroundings for a wide variety of purposes, most notably human and veterinary medicine, cosmetics, hedging, fencing, brewing, weaning children from breast-feeding and in traditional rituals (Bjorå *et al.*, 2015; Grace *et al.*, 2009; Schmelzer *et al.*, 2008; Wabuyele, 2006). With respect to Tanzania, Amir *et al*. (2019) recorded thirty-seven uses of *Aloe* species in four major categories: Human medicine, livestock (veterinary) medicine, cosmetics and other non-specific uses not relating to health and well-being. Tanzania, a country rich in *Aloe* diversity, has 52 *Aloe* species, 24 of which are endemic to the Tanzanian mainland and two in Zanzibar (Reynolds, 2004; Wabuyele & Kyalo, 2008). Though some species are quite common, almost one quarter (23%) are threatened, while the current population trend of specific species is unknown (Wabuyele *et al.*, 2008). For instance, it is asserted that among the five CR Tanzanian *Aloe* species, *A. boscawenii* has not been sighted or collected since 1953 (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b). Other CR species are *Aloe dorotheae*, *Aloe flexilifolia* Christian, *Aloe leptosiphon* A. Berger and *Aloe pembana* L. E. Newton while many others are in the threatened categories EN and VU. This literature review will highlight issues concerning the IUCN Red Listing of *Aloe* species, threat factors and prospects for conservation.

2.3 The International Union for Conservation of Nature Red List and Threatened *Aloe* Species

The categories (Fig. 3) and criteria for the IUCN Red List were first published in 1994 as explained in Appendix 1 (IUCN Standards and Petitions Subcommittee, 2014). The Convention on International Trade in Wild Species of Flora and Fauna lists *Aloe* species in Appendices I and II (CITES, 2021). In general, the rate at which the species of both flora and fauna worldwide are declining is alarming (Djoghalf, 2007). It is estimated that one plant species become extinct for each passing day due to various factors, including human activities (Akeroyd, 2002). The genus *Aloe* has been one of the most traded medicinal plants and known in the world market trade and cultivation for thousands of years. During the mid-19th century, East Africa was one of principal exporter *Aloe* species, i.e., Socotrine and Zanzibar aloe. Socotrine aloe ranked as a vital materia medica of Dispensatory of the United State (Haller, 1990).

In Tanzania, the *Aloe* species are declining rapidly, with five species being categorized as CR, and several threatened due to limited distributions, small population size and human activities including over-harvesting (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009h, 2009f, 2009d, 2009e, 2009b).

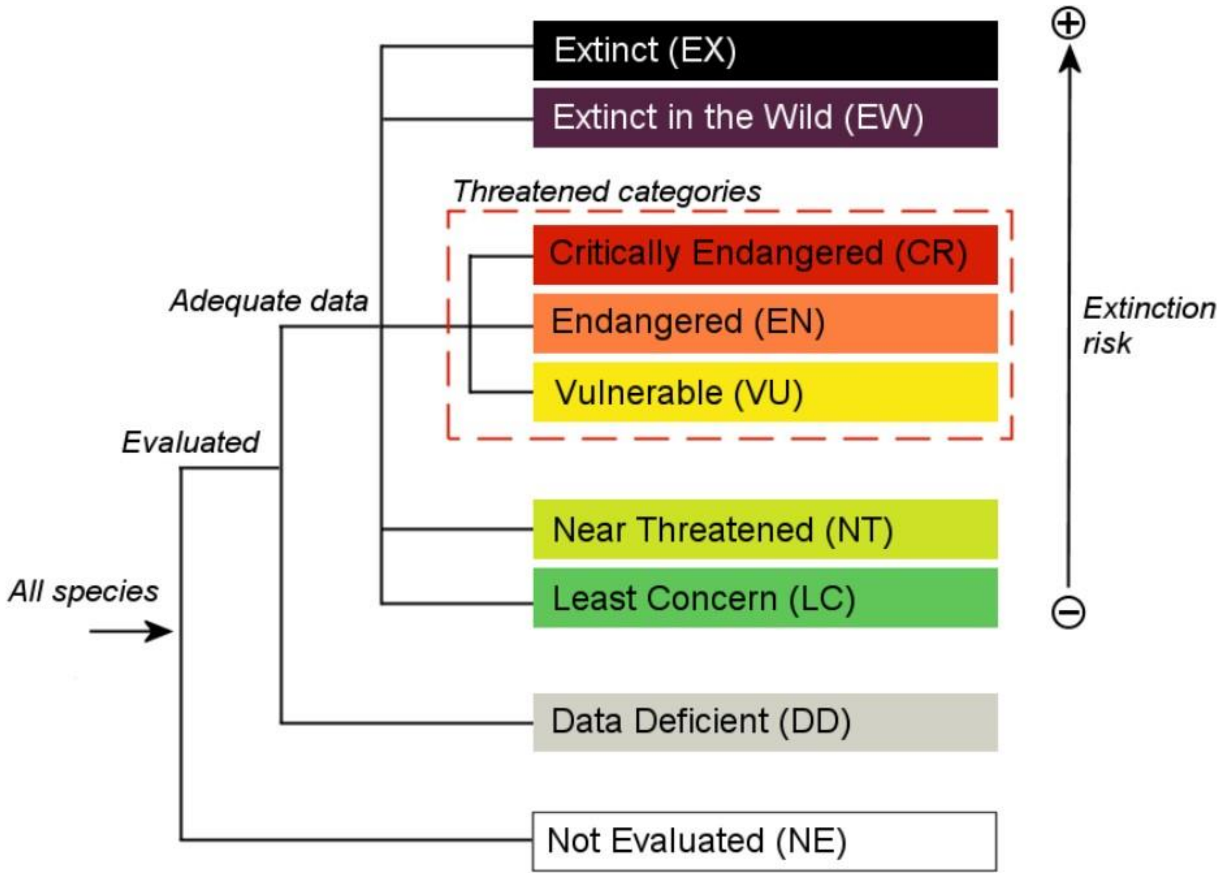

Figure 3: Structure of the The International Union for Conservation of Nature Red List Categories, (IUCN Standards and Petitions Subcommittee, 2014)

Aloe boscawenii is one of the CR *Aloe* species in Tanzania, allegedly having not been sighted in the wild since 1953, despite a serious effort to trace it (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b). The remaining CR *Aloe* species, i.e., *Aloe dorotheae*, *Aloe flexilifolia*, *Aloe leptosiphon* and *Aloe pembana* all have small EOOs (EasternArc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009h, 2009f, 2009d, 2009e).

Tools used in the IUCN assessment of threatened species include IDRISI for Windows Geographic Information System package created for spatial analysis and map production (United Nations Environment Programme [UNEP], 1995) as well as RAMAS® Red List (Version 2.0) which categorizes the species (Pfab and Victor, 2002), GIS based on modelling such as BIOMAPPER (Lobo & Chefaoui, 2005) and ArcGIS (Kutnar & Hladnik, 2016). The mentioned tools require proficiency in computation, are expensive and time-consuming. This called for more assessment techniques that would be quick, cheap and user-friendly (Bachman *et al.*, 2011).

The GeoCAT was used in this study, analysed geospatial information based on criterion B, i.e., geographic range in the form of EOO and/or AOO (Bachman *et al.,* 2011). The EOO is a measure of the geographic range size of a species while AOO is a measure of the area in which a species occurs (Abeli *et al.,* 2009; Brummitt *et al.,* 2008; IUCN Standards and Petitions Subcommittee, 2014). The two-sub criteria of criterion B are B1 (EOO< 100 km^2 for CR < 5000 km^3 for EN and < 20 000 km^3 for VU) and B2 (AOO< 10 km^2 for CR < 500 km^2 for EN and < 2000 km^2 for VU) (Table 1) (Bland *et al.,* 2016; IUCN Standards and Petitions Committee, 2019). The online data such as GBIF, Flickr and Scratchpads can be combined with the field generated information to investigate particular species' threat status (Bachman *et al.,* 2011). Because it uses the Google Maps API and the custom user interface to provide data locations (Bachman *et al.*, 2011) it also produces quality maps. Other criteria used for assessment by the IUCN include criterion A, i.e., population reduction, criterion C, i.e. small population size and decline, criterion D, i.e., very small or restricted population and criterion E, i.e. quantitative analysis (IUCN Standards and Petitions Committee, 2019). A fully assessment using Criteria B and C for CR or Vulnerable Criteria D should be assessed for a taxon known only from its type of location and face a substantial threat (IUCN Standards and Petitions Committee, 2019).

2.4 Seed Germination of *Aloe* Species

Due to most *Aloe* seeds' morphology, they can be dispersed by wind (Cousins *et al.,* 2013, 2014; Symes, 2012; Wilson *et al.,* 2009). Additionally, there is a possibility that *Aloe* species can be ingested and spread by wild animals, birds and even insects (Arena *et al.,* 2013; Bani *et al.,* 2016; Patil *et al.,* 2017; Traveset *et al.,* 2001). When the seeds pass through the intestines of the host organism, their viability is improved by the exposition to the embryo (Barnea *et al.,* 2006; Kleyheeg *et al.,* 2018; Traveset *et al.,* 2001). A better germination percentage was observed by *Asparagus acutifolius* L. and *Osyris alba* L. when ingested by *Turdus merula* and *Sylvia melanocephala* bird species (Traveset *et al.,* 2001). For the *Aloe* species, their interaction with opportunistic birds (Arena *et al.,* 2013; Payne*et al.,* 2019) and mouse (Payne *et al.,* 2019) improved pollination and seed viability of *Aloe Peglerae* Schoenland.

The LC species such as *Aloe lateritia* Engl., *Aloe volkensii* Engl. and *Aloe secundiflora* Engl. (according to the IUCN Red List) are broadly available across Tanzania (Carter, 1994). This explains their adaptability to several environmental conditions (Brown, 1984). Contrarily, the rare and endemic coastal *Aloe* species, particularly CR *Aloe boscawenii* Christian and *Aloe pembana* L. E. Newton (Campbell-Barker, 2012; Carter, 1994; Eastern Arc Mountains & Coastal Forests CEPF

Plant Assessment Project Participants, 2009b; Letsara *et al.*, 2012) might be inadaptable to varying environmental conditions. Deficient of nutrient (Willis & Chapman, 2006), salinity condition (Kahn & Durako, 2005; Willis & Chapman, 2006) and sea-level rise (Araujo & Pereira, 2007; Calvão *et al.*, 2013) influence the low performance of the endemic coastal plants. Changing temperature can also affect low germination and poor seedling establishment (Kulkarni *et al.*, 2006; Teketay, 1996).

2.5 Sexual Propagation

Species in the genus *Aloe* are self-incompatible in the breeding system (Botes *et al.*, 2009; Cousins *et al.*, 2013; Cristiano *et al.*, 2016). The genus requires cross-pollination for the seeds to be fertile. This results in the heterogeneity of seedlings (Cristiano *et al.*, 2016) except for few species such as *A. peglerae* Schönland (Arena *et al.*, 2013), *Aloe kraussii* Baker and *Aloe maculata* All., where both self and cross-pollination are possible. This method, i.e., the sexual process encompasses meiosis, resulting in genetic variability within the population (Cabahug *et al.*, 2018). Hence, the need to apply technologies such as tissue culture to support the homogeneity of the seedlings (Engelmann, 2004) and combat the effects of self-incompatibility when pollinator species are unavailable (Genena *et al.*, 2008; Pande & Gupta, 2013).

2.6 Asexual Propagation

This involves propagation through cuttings and in-vitro culture. Through this method, the plant's genetic integrity is maintained (Sarasan *et al.*, 2011; Wochok, 1981).

2.6.1 Propagation through Cuttings

Propagation of an *Aloe* is mostly done through cutting the offsets/ pups/ suckers from the mature plants (Cabahug *et al.*, 2018; Cristiano *et al.*, 2016; Farrokhi *et al.*, 2017). For genus *Aloe*, the mature individual gives a minimum of three up to four pups for a season. This is the alternative for the continuation of the species when self and cross-pollination does not take place. The propagation method through cuttings of the stem and leaves is challenging due to possible fungal infection of the wound (Farrokhi *et al.*, 2017). For all the propagation methods, the seedlings watering should be moderate, and the soil should be allowed to dry between watering periods (Farrokhi *et al.*, 2017). The stem cuttings should have at least two buds of about 5 to 6 cm long and be placed in the prepared containers (Yousafzai *et al.*, 2012). Eighteen months after planting, the *Aloe* will be ready for harvest (Cristiano *et al.*, 2016).

2.6.2 In-Vitro or Tissue Culture

Tissue culture can regenerate and propagate plants from single cells, tissue or organ in a controlled setting (Gupta, 2014). The technology has been famous in the germination of ornamental plants for horticulture business (Wochok, 1981). This technology gives disease-free clones (Abadi & Kaviani, 2010; Curvetto *et al.*, 2006; Wochok, 1981), in large scale (Fay, 1994; Abadi & Kaviani, 2010) and a shorter period of multiplication (Fay, 1994; Wochok, 1981) with conservancy of germplasm (Sarasan *et al.*, 2011; Wochok, 1981). The traditional propagation method is challenging when plant materials are scarce, for example, the rare and endemic species, tissue culture can be appliedto regenerate the population (Wochok, 1981).

The technology has been studied in the conservation of threatened species such as *Gentiana kurroo* Royle (Sharma *et al.,* 1993), *Withania coagulans* (Stocks) Dunal (Jain, 2009) and *Dianthus superbus* L. (Mikulík, 1999). Due to the increasing worldwide market demand for *Aloe* and its products, tissue culture is now applicable to *Aloe* species' propagation (Cristiano *et al.,* 2016). However, few *Aloe* species have practically multiplied from this method both for conservation and for commercial purposes. Examples of commercial propagation of *Aloe* species tissue culture includes *A. ferox* (Bairu *et al.*, 2009) and *Aloe vera* (L.) Burm. F (Gupta, 2014; Natali *et al.*, 1990). Studies that aim for conservation purposes include *Aloe* species such as *Aloe polyphylla* Pillans (Abrie & van Staden, 2001).

The *Aloe* explant material that has mostly being propagated through tissue culture includes shoot tip or apical meristem tip (Amoo *et al.,* 2012; Abadi & Kaviani, 2010; Gupta, 2014; Natali *et al.,* 1990; Nayanakantha *et al.,* 2011; Sharma *et al.,* 2010), nodal (Gupta, 2014; Jain *et al.,* 2009; Roy & Sarkar, 1991; Sharma *et al.,* 1993), leaves (Gupta, 2014), as well as leave sheath (Castorena Sanchez *et al.,* 1988). Surface sterilization in tissue culture is mostly done using mercuric chloride (Bairu *et al.,* 2009; Aggarwal & Barna, 2014; Gupta, 2014; Roy & Sarkar, 1991; Sidhu, 2010) and sodium hypochlorite (Kawai *et al.,* 1993).

Plant growth regulators are crucial for the shoot and root formation in in-vitro propagation (Amoo *et al.,* 2012; Chukwujekwu *et al.,* 2002; Liao *et al.,* 2004; Sultan *et al.,* 2006). The eminent proliferation plant growth regulators for *Aloe* species includes kinetin (Chukwujekwu *et al.,* 2002; Kawai *et al.,* 1993; Natali *et al.,* 1990; Roy & Sarkar, 1991), benzyl adenine (BA) (Abrie & van Staden, 2001; Adelberg & Naylor-adelberg, 2012; Abadi & Kaviani, 2010; Sharma *et al.,* 1993), zeatin (Chukwujekwu *et al.,* 2002) and Indol-3-butyric acid (IBA) (Abadi & Kaviani, 2010). The most common used rooting plant growth regulator includes IBA (Abadi & Kaviani, 2010), however, in some studies rooting was done in the absence of plant growth regulators (Aggarwal & Barna, 2014).

The main challenge with in-vitro culture is the browning phenomenon which results from phenolic compounds present in the explant (Abrie & van Staden, 2001; Nayanakantha *et al.*, 2011). Polyvinylpyrrolidone (PVP) (Liao *et al.*, 2004; Roy & Sarkar, 1991), ascorbic acid (Abadi & Kaviani, 2010), activated charcoal and citric acid (Abadi & Kaviani, 2010; Nayanakantha *et al.*, 2011) are the primary reagents utilized in controlling phenolic browning in in-vitro culture. Furthermore, one of the limitations of tissue culture is the constraint of production. For example, the compound taxol in *Taxus* species takes 60 years of age to achieve higher yield, whereas tissue culture in a controlled environment is harvested after 6 years (Isah, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethnobotanical Knowledge and Assessment of *Aloe* Species in Tanzania

3.1.1 Study Area

Information on the ethnobotany, occurrence, distribution and threat to the *Aloe* species was collected from December 2017 to August 2018 from Kilimanjaro, Tanga, Mara (Serengeti district) and Katavi-Rukwa regions of Tanzania as depicted in Fig. 4. Except for Serengeti district, the chosen areas are among the Tanzanian *Aloe* diversity (Carter, 1994). Serengeti district was added as no findings were reported before. The research clearances were obtained from respective district authorities.

3.1.2 Sampling Technique

Ethnopharmacological studies were used as the standards in gathering and reporting ethnobotanical data (Weckerle *et al.*, 2018). Ethnobotanical knowledge was collected from 22 villages in areas of known high *Aloe* species diversity (Fig. 4). For the wildly available species, four to eight wards were selected and for the species with small distribution range and endemic to a specific area, only such locations were selected, i.e. endemic *Aloe* species.

Respondents were selected randomly to capture an extensive range of ethnicities, gender, ages and occupations (traditional healers, village leaders, teachers and villagers). The ethnobotanical knowledge from local respondents was obtained through a semi-structured interview. A total of 236 respondents were interviewed from 22 villages using the sample size formula below:

$n = (Z\sigma/E)^2$, where Z = 1.96; n, σ and E is the sample size, standard deviation and margin of error, respectively.

Important detailed information such as folk taxonomy, flowering season, estimated access to the species, phenology, parts of plant utilized, harvesting methods, preparation, use, potential side effects and possible threat factors were gathered. The questionnaires were supplemented by participatory field visits by the help of local informants. Photos and direct field observations were used to confirm the folk taxonomy mentioned for the available species in the wild. Voucher specimens were collected from each site and later deposited at the National Herbarium of Tanzania

(NHT)- Tropical Pesticides Research Institute (TPRI) in Arusha, at the Institute of Traditional Medicine herbarium (ITM)- The Muhimbili University of Health and Allied Sciences (MUHAS), and at the herbarium of the University of Dar-Es-Salaam (DSM). As suggested by Carter (1994), the morphological identification of *Aloe* species followed the Flora of Tropical East Africa, *Aloaceae* and experts (botanist at the NHT) was consulted if the identification was difficult.

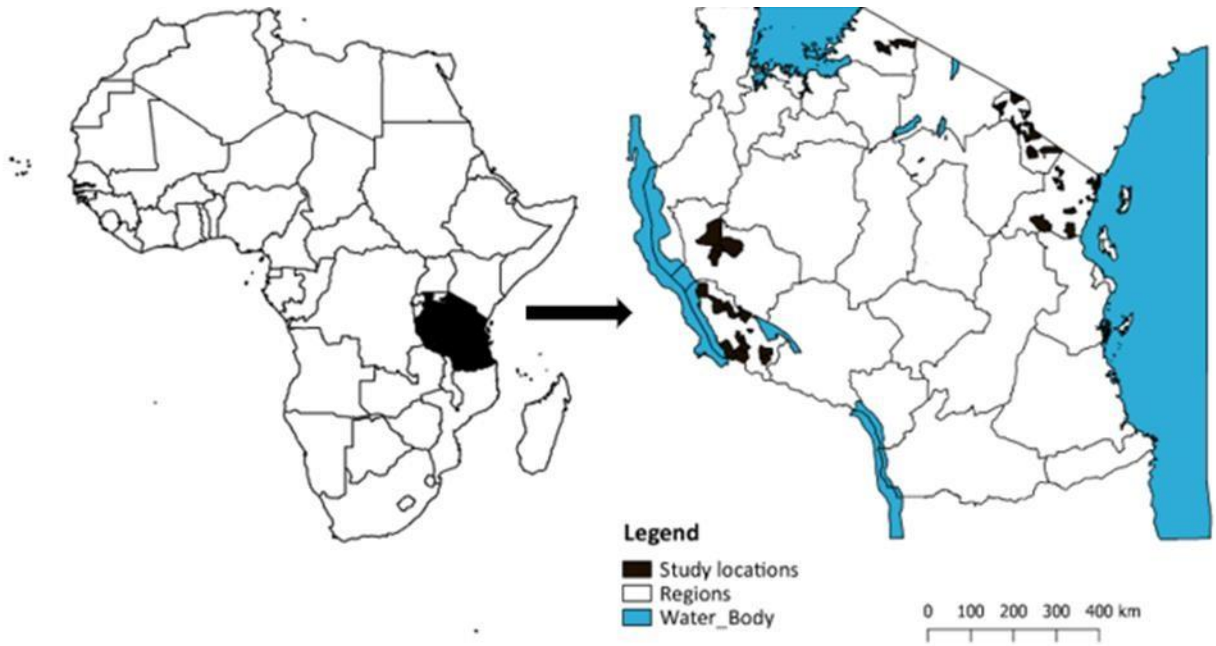

Figure 4: The map of Africa and Tanzania, the shaded areas show the locations where interviews took place for eighteen districts on *Aloe* species distribution and use between the years 2017 and 2018

To understand the distribution of different *Aloe* species, a total of 22 regions (Kilimanjaro, Tanga, Katavi, Rukwa, Mara, Arusha, Manyara, Dodoma, Singida, Iringa, Morogoro, Pwani, Dar Es Salaam, Njombe, Lindi, Mtwara, Ruvuma, Mbeya, Tabora, Shinyanga, Mwanza and Zanzibar) were assessed in a stratified random meander survey (Huebner 2007; Mccaffrey *et al*., 2014). For the species with consistent habitat preference in a landscape, especially those growing along water bodies and seashore, a systematic search was conducted within the specific site (Mccaffrey *et al*., 2014). Colleagues working in areas other than the study sites assisted in bringing the individual *Aloe* and coordinates where it was sourced. Coordinates and elevation were determined and later mapped the *Aloe* species' distribution and diversity across the study sites using ArcGIS version 10.4 (Yan *et al*., 2020). The use of nation herbarium databases complemented mapping, i.e., NHT and DSM, as well as international herbarium databases, i.e., Royal Botanic Gardens Kew (K), Missouri Botanical Garden (MO), Swedish Museum of Natural History (S), Desert Botanical Garden (DES), Naturalis (WAG) and Aarhus University (AAU). The duplicate voucher specimens were collected and deposited at the nation herbaria: ITMH, DSM and NHT (Table 2). Community interviews supplemented the distribution mapping to evaluate the communities' view on what threatened local *Aloe* species.

3.1.3 Data Analysis

Data on the ethnobotanical information were analyzed using SPSS v.20 (Sarper *et al.,* 2009). Data were first tested for normality and equality of variance with Shapiro-Wilk and Kolmogorov-Smirnov tests at $\alpha = 0.05$ (Srithi *et al.,* 2009). Ethnobotanical knowledge (number of uses of *Aloe* species named by each respondent) and the relationship between age groups, gender, and whether the perception of *Aloe* species' possible disappearance was age-dependent were analyzed using Mann-Whitney U-tests. The link between perception on factors affecting *Aloe* species abundance and the number of *Aloe* species used was tested using the Kruskal Wallis Test (Number of uses = ethnobotanical knowledge).

3.2 International Union for Conservation of Nature Red List Categorization of *Aloe* Species in Tanzania

Red List categories were assigned to the different *Aloe* species following the guidelines set by the IUCN-SSC (IUCN Standards and Petitions Subcommittee, 2014) (Table 1). This Red List assessment applies to *Aloe* species endemic to Tanzania at both the national and international levels. However, they only apply at the national level in Tanzania for *Aloe* species found in countries other than Tanzania.

The GeoCAT was used to analyze geospatial information based on the requirements B, i.e., restricted geographical range with two sub-criteria, the EOO, the AOO (Bachman *et al*., 2011) and the number of locations. The number of locations is considered to be an indicator of a significant potential threat(s) that could reduce or eliminate the population (IUCN Standards and Petitions Committee, 2019). Criteria C and D were not applied because there was insufficient information on population data, and there was no estimated population decrease compared to the previous population for Criteria A. *Aloe* species were categorized according to their threatened status based on the IUCN Red List criteria (2011) (Table 1), i.e. CR, Endangered (EN), Vulnerable (VU), or Nearly Threatened and LC. Species were categorized as Data Deficient in case data was insufficient. For the species that fell under the threatened category, particular CR, EN and VU, sub-criteria (Table 1) were added in the assessment. Moreover, species were locally mapped, and the current or potential threat factors for the species were determined within their EOO.

3.2.1 Data Analysis

Descriptive statistics were performed using IBM SPSS Statistical package v. 20 (Awang *et al*., 2018) to determine respondents views on *Aloe* species' threat level and conservation practices. In each assessed region, Pearson's correlation analysis determined the correlation between elevation and diversity of *Aloe* species.

Table 1: The Extent of Occurrence (EOO) and the Area of Occupancy that were used for assessing the current status of the threatened *Aloe* species in Tanzania

Geographic range	Critically Endangered	Endangered	Vulnerable
B1. EOO	< 100 km^2	< 5000 km^2	< 20 000 km^2
B2. AOO	< 10 km^2	< 500 km^2	< 2000 km^2

And at least two of the following: Severely Fragmented OR Number of locations, Continuing Decline and Extreme Fluctuations were added in the assessment.

3.3 Propagation of the Critically Endangered and Least Concern *Aloe* Species

3.3.1 Seed Collection

A total of five Tanzanian *Aloe* species seeds were collected from wild populations (Table 2). The seeds were stored before trials so that the experimentation is conducted simultaneously. Consideration was given because the storage of seeds at room temperature in a dry place allows a higher germination percentage for *Aloe* seeds (Cousins *et al.,* 2013). Moreover, seeds viability was check immediately after collection and compared with the finally experiment and there was no any significant difference.

Table 2: Accession location, storage time (in months), and flowering months for the *Aloe* species that were germinated in this study

IUCN Red List Status	*Aloe* species	District	Accession locations	Storage time	Flowering months
Critically endangered	*A. pembana*	Pemba	5°21'S, 39°38'E	2	Nov – March
	A. boscawenii	Mkinga, Tanga	4°49'S, 39°6'E	5	
Least Concern	*A. lateritia*	Handeni, Tanga	5°25'S, 38°1'E	11	
	A. secundiflora	Same, Kilimanjaro	4°15'S, 37°55'E	7	May – Aug Nov – Feb
	A. volkensii	Serengeti, Mara	1°50′S, 34°40′E	14	

3.3.2 Experimental Design

The traditional seed germination experiments were performed at the Nelson Mandela African Institution of Science and Technology laboratory in Arusha, Tanzania. The process involved sorting *Aloe* seeds by hand to obtain the undamaged seeds, enabled by the fingers' touch to feel the embryo. The presence of embryo hinted that seeds were healthy and suitable for the experiment (Cousins *et al.,* 2014). The effect of light condition was evaluated concerning scarification, temperature variation, growth medium, varying concentrations of salt and potassium nitrate on seed germination of the chosen *Aloe* species; the details on different treatments are explained in Table 3. *Aloe* seeds' sterilization (960 seeds per *Aloe* species) was done by washing with 2% sodium hypochlorite for 15 minutes, then rinsing with distilled water three times (Arena *et al.,* 2013). The *Aloe* seeds were positioned in a filter paper (except for the soil treatment) and sprayed with the respective solution every two days. Each petri dish contained 20 seeds and replicated four times (Çavusoğlu *et al.*, 2016), culminating in 80 seeds for each parameter. Germination of seeds was documented daily, and successful germination was established when the radicle length was equal to 2 mm. The experiments were completed when no new seeds sprouted after 21 days since planting. For saline water, no germination was observed after 21 days. The seeds that did not germinate were washed three times before the distilled water was applied in the new filter paper for 21 days. The subsequent seedlings for all treatments were planted in soil mixed with gravel to monitor their growth and later were handed to the NM-AIST and TPRI in Arusha, Tanzania.

3.3.3 Data Analysis

Data were tested for normality using Shapiro-Wilk and Kolmogorov-Smirnov test at a significance level of $\alpha = 0.05$. The Final Germination Percentage (FGP = final number of seeds that germinated divided by the total number of seeds × 100) (Kader, 2005) was calculated for all treatments. A two-factorial ANOVA was applied to evaluate each treatment's main effect for the different *Aloe* species and their interaction effect on seeds germination. *Aloe* species (20 seeds per petri dish) and treatments were assigned as fixed factors while the response variable was the probability of FGP. Similarly, the FGP among *Aloe* species was compared in all treatments by testing the differences using an ANOVA, followed by Tukey's post hoc tests (Hamasha & Hensen, 2009; Le Stradic *et al.*, 2015). Student t-test was used to compare the differences in germination initiation between seeds germinating in distilled water medium and the seeds recovered to distilled water media after saline water was used. Further, t-test was used to determine the difference between the seeds grown on soil medium and those on filter paper. The significance level was set at $\alpha = 0.05$ and OriginPro 2015 statistical package (Upadhyay & Mishra, 2015) was used for analysis.

Table 3: Different treatments that were used in the experiment to mimic the environmental conditions and current or possible future climate change effects. n = 80 per treatment, N = 960 per *Aloe* species (DW = Distilled water, FP = Filter paper, D&N = Day and night, N = Night)

Environmental condition	Treatments	Media	Temp. (°C)	Light	Representation
Constant temperature of seeds in treatments	25°C- Low (control)	FP and DW	25	D&N	Average temperature of the regions where the species were found.
	30°C- Moderate	FP and DW	30	D&N	
	35°C- High	FP and DW	35	D&N	Simulating the effect of global warming in Tanzania (Luhunga *et al.*, 2018).
Light treatment	Light (control)	FP and DW	25	D&N	Shade generated by older plants (Giddy, 1973).
	Dark	FP and DW	25	N	Seeds lying beneath the ground (Kazuhiko *et al.*, 1997).
Scarification treatment	Scarified	FP and DW	25	D&N	Outer wing removed (except for *A. pembana*), coat removed at a distal end (Abrie & van Staden, 2001; Clemens *et al.*, 1977) to allowing penetration of water into an embryo (Copeland & McDonald, 1999).
	Un-scarified (control)	FP and DW	25	D&N	No interference
Effect of KNO_3	0 mg/L (control)	FP and DW	25	D&N	Coastal soils (referred to soil with present of *Aloe* adjacent to the ocean) contain excess salt, which decreases nutrient uptake through increasing osmosis (Kulkarni *et al.*, 2013; Moghbeli, 2012).
	0.01 mg/L	FP and KNO_3	25	D&N	
	0.1 mg/L	FP and KNO_3	25	D&N	
Salt treatment	Salt plus water	FP and saline water	25	D&N	Coastal *Aloe* species (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009h, 2009b). Salinity tends to reduce germination success (Jin *et al.*, 2007; Moghbeli, 2012; Murillo-Amador *et al.*, 2014).
	DW (control)	FP and DW	25	D&N	If sea spray disappears
Soil treatment	DW (control)	FP and DW	25	D&N	
	Soil	FP and Soil (soil from where the plant occur natural)	25	D&N	Gravel providing support for poorly established roots of *Aloe* seedlings (Hankey & Smith, 2006; Pascaline *et al.*, 2010). The soil was collected 10 cm depth from the respective *Aloe* location and mixed with gravel at ratio 1/3 (gravels) and 2/3 (soil). Aloe were tested on respective sample soil.

3.4 Tissue culture

3.4.1 Study Site and Soil Sample Collection

An intensive systematic search was conducted along the coast of Mkinga district in Tanga region in wards were *A. boscawenii* was previous reported in Tanzania. Coordinates were taken from the four locations in the wild and one in cultivation, where *A. boscawenii* were sighted (Fig. 5). Soil samples were collected from where they were removed before the collection of soil samples. The soil collection was taken from the middle and the four angles of the quadrats established at 10 cm depth, latter, combined to make a single collection from each site. The soil samples were placed in zip-lock plastic bags and taken for analysis to determine different soil parameters at Seliani Agricultural Research Institute, Arusha Tanzania.

The sieving of soil to get rid of fine rocks, roots, and other unwanted particles performed using 2 mm fine mesh screen. The analysis of soil for chemical properties were as follows: Organic carbon (OC), pH, Electrical conductivity (EC), Organic matter, Phosphorus (P), Calcium (Ca), Total nitrogen (Total N), Potassium (K), Magnesium (Mg), Manganese (Mn) and Cation exchange capacity (CEC). Total OC was evaluated using the Tinsley technique; the pH was obtained potentiometrically in a soil suspension, EC was determined in soil suspension by Walkley-Black both in ratio1:2.5, and Kjeldahl methods determined the total N from the soil; extraction of P was measured using 0.5 M $NaHCO_3$ with the ascorbic acid molybdate method; The soil cation, i.e. Ca^{2+}, Mg^{2+} and K^+ were extracted using $NH_4C_2H_3O_2$ and evaluated on atomic absorption spectrophotometer. For the Mn, and CEC, were obtained using diethylene triamine pentaacetic acid (DTPA) and later determined with Ammonium Acetate method at pH 7.0.

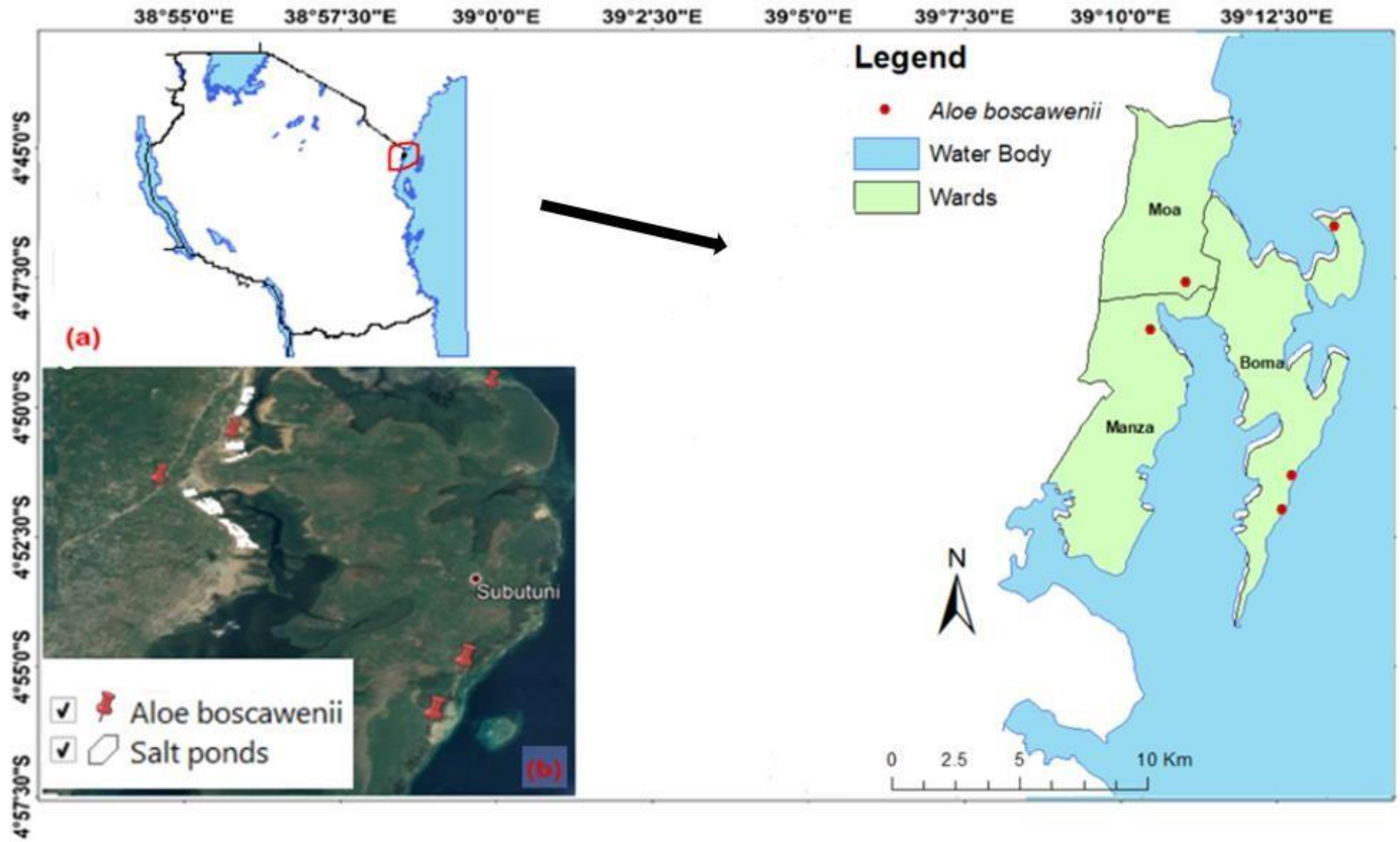

Figure 5: Map showing (a) Tanzania with the wards where *A. boscawenii* is found, (b) locations of *A. boscawenii* close to the salt ponds and (c) *Aloe boscawenii* access points in three wards in Mkinga District, Tanga region

3.4.2 Plant Materials and Sterilization

Samples for tissue culture were collected from healthy individual plants in four access population as representatives of the entire populations (estimate of each subpopulation, N $\geq$ 50). Explant materials (4 cm) were taken from leaf tip (Gupta, 2014), sheath (Sanchez *et al.,* 1988), roots, meristems (Amoo *et al.,* 2012; Gupta, 2014; Abadi & Kaviani, 2010) and also from seeds (Groenewald *et al.,* 1975). The explants were first washed in running water for 20 min before being sterilized with 70% ethanol for 1 minute (Abrie & van Staden, 2001; Dorman *et al.,* 2009). The performance of both Sodium hypochlorite (NaOCl) (Abrie & van Staden, 2001; Dorman *et al.,* 2009; Abadi & Kaviani, 2010) and Mercury nitrate ($Hg(NO_3)_2$) in surface sterilization were tested to evaluate their performance. Explant materials were soaked in three replicates of different concentration of NaOCl (3%, 4%, 5%, 6%, 7% and 10% w/v) and 0.1 % w/v $Hg(NO_3)_2$ then mixed with two drops of tween 20 (for better penetration of NaOCl and $Hg(NO_3)_2$ for 30 minute and 15 minutes respectively. The samples were later rinsed with distilled water three times. In concentration that performed better (6% NaOCl), 0.1% g/l Antifungal (Metalaxyl 8% + Mancozeb 64%) was incorporated into the media to control fungal contamination as it was for antibiotic (Msogoya *et al.,* 2012; Reed & Tanprasert, 1995) and antifungal (Msogoya *et al.,* 2012) in other studies.

3.4.3 Media and Culture Conditions

The media was prepared by modifying the Murashige (1974) and Murashige and Skoog (1962) protocol. After a thorough surface sterilization, the ends of explant materials were trimmed and clipped into small pieces (~0.5 cm^2) to fit into the culture vessels. The *A. boscawenii* samples were aseptically cultured in a culture vessel containing 20 mL of culture. The media culture contained MS basal salts supplemented with 30 g/L sucrose, 100 mL microelement, 10 mL Fe-EDTA, 5 mL microelement, 5 mL vitamins; 5 mL ascorbic acid, 5 mL BAP and solidified with 4 g/L of agar. The pH was adjusted to 5.8 ± 0.1 before autoclaving at 121˚C for 15 min. The cultured samples were maintained at a temperature of 25 ± 2˚C, 16 h photoperiod with 3000 lux of light intensity (Molsaghi *et al.,* 2014).

The culture was sustained by sub-culturing five times at an interval of 6 weeks to increase the number of invitro buds (Baskaran *et al.,* 2015; Kiran *et al.,* 2017; Molsaghi *et al.,* 2014). The in vitro regenerated elongated shoots (3 to 4 cm) were removed aseptically and implanted on half potency MS medium (same as shooting media). Different concentration of rooting hormones, NAA and IBA and their combination were used in rooting although *A. barbadensis* (Sanchez *et al.,* 1988; Natali *et al.*, 1990) and *A. ferox* (Bairu *et al.,* 2009) perform best on hormone free medium. A total of five plantlets (3 replicates per treatment) were carefully washed, measured and pre-acclimatized in tray enclosed in different hardening media soil for two weeks and later opened to grow for another six weeks. The trays were enclosed to provide moisture, humidity, and required temperature for the plantlet to survive outside (Mazri, 2012). The primary acclimatization was tested in different soil medium including; sand, sawdust, coco peat and mixture of sand and sawdust (1:1), sand and coco peat (1:1) (Cristiano *et al.*, 2016) and sand, sawdust and coco peat (1:1:1) were used to test survival and multiplication rate of the plantlets during the acclimatization process. Due to coco peat being not always available and expensive (Cristiano *et al.*, 2016), replacing it or mixing with other material will reduce its uses. The sawdust has never been reported to be used for acclimatization of any *Aloe* species. The plant survival rate was analyzed after eight weeks and then transferred to the soil (secondary acclimatization) and kept in the greenhouse for growth. The rooted explants were watered every two days interval and later restored at the different site where it was previously found.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Ethnobotanical Knowledge of *Aloe* Species

A total of 236 respondents were interviewed, 91 in Tanga, 22 in Mara (Serengeti district), 56 in Kilimanjaro and 67 in Katavi-Rukwa regions. The youngest and oldest respondents were 18 and 85 years old, respectively (Fig. 6). The education level of most respondents was primary education (70%), followed by lower secondary education (Ordinary Level) (14%) and then no formal education (13%). Majority of the respondents were farmers (89%) followed by Entrepreneur (6), fishermen's (2), breeders, teachers and students. Most respondents had lived in the respective area for 10 to 30 years. Respondents spoke different local languages based on their tribe: Digo, Fipa, Sambaa, Zigua, Bena, Muarusha, Kamba, Maasai, Meru, Pare, Chaga, Kurya, Lungu, Gongwe, Pimbwe, Sukuma and Nyiha.

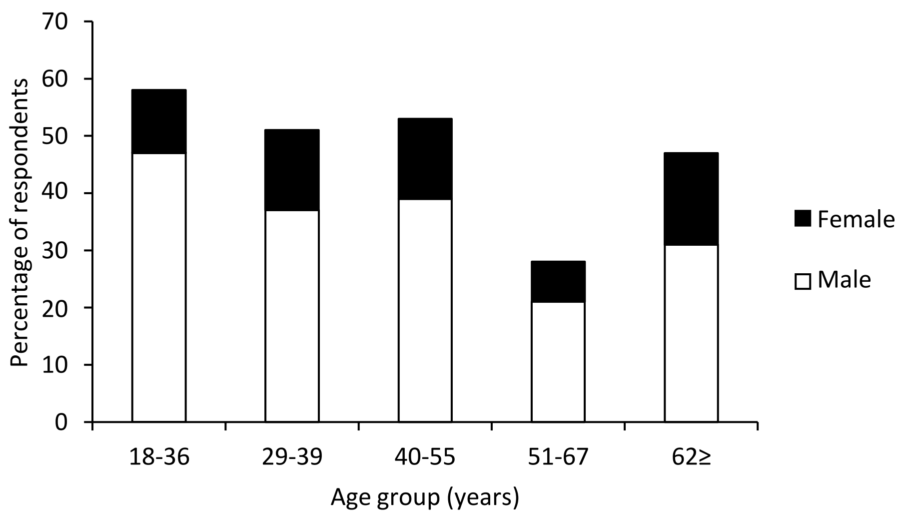

Figure 6: Age and gender distribution of all respondents (n = 236) across the four study regions within Tanzania that were asked about *Aloe* species distribution and their use

4.1.2 Folk Taxonomy and Flowering Times of *Aloe* Species

The respondents knew different *Aloe* species and 93% (n = 221) declaring *Aloe vera* is the common name for all the *Aloe* species and distinguished them by giving each a folk name in their local language (Appendix 2). Respondents (64%, n = 151) were able to differentiate various species based on morphological features, particularly leaf spots (62%), shoot height (50%), leaf size (19%), leaf colour (10%), the taste of the sap (3%), and habitat in which they were found (3%). In case of Pare tribe in Kilimanjaro region, they called all the *Aloe* species *Kithapa* but distinguished the short and tall *Aloe* species, "*Kithapa kidori*" and "*Kithapa kibaha*", respectively. Furthermore, they differentiated species based on their use, i.e., "*Kithapa cha vujawa*" (*Aloe* species for beer brewing). This local drink *"vujawa"* is made from fermenting sugarcane and *Aloe* stems "*miatini*". The *Aloe* stem is added to give a pleasant bitterness to the beer. People from Tanga region distinguishes the *Aloe* species based on their locality. For example, the Sambaa tribe referes to "*Kovongo ja makungu*", specifically for the *Aloe* from the rocks and based on their colour "*Kovongo ikundu*", meaning Aloe which turn red during the dry season and green during the rainy season. They also have a particular Aloe "tall Aloe" they called "*Mauza*" planted along the graveside to separate different periods in which their ancestors lay in the grave. The morphologically similar species were treated as the same species and used interchangeably, for example, the "tall Aloes" *A. ballyi* Reynolds and *A. volkensii* Engl. and the "short Aloes" *A. rabaiensis* Rendle and *A. deserti* A. Berger, both in the Kilimanjaro region. The remaining 36% of the respondents claimed to have only one *Aloe* species in their area, but more than one species was observed during the field surveys.

The *Aloe* species gives flower immediately after the rainy season but fluctuated based on the region and the species' nature (Fig. 7). Most species tend to flower in June to August in Kilimanjaro, Tanga and Katavi-Rukwa. In Serengeti district, Mara they were observed to flower in March after the short rain season. Nevertheless, *A. boscawenii*, *A. leptosiphon* A. Berger and *A. massawana* Reynolds, flowered from January to March in Tanga region.

4.1.3 *Aloe* Species Uses by Local Communities

There was no difference in ethnobotanical knowledge between women and men (Z = 0.712, p = 0.476). The old (42-85 years) had a higher level of ethnobotanical experience than the youth (18 to 41 years; Z = 2.144, p = 0.032). The knowledge was transferred mostly by the male gender, especially fathers (40%) and grandfathers (29%). The information was mainly on where to find and how to use the *Aloe* species. The Aloes were highly utilized during the seasonal outbreak of malaria in humans and Newcastle disease in chickens, following the rainy season.

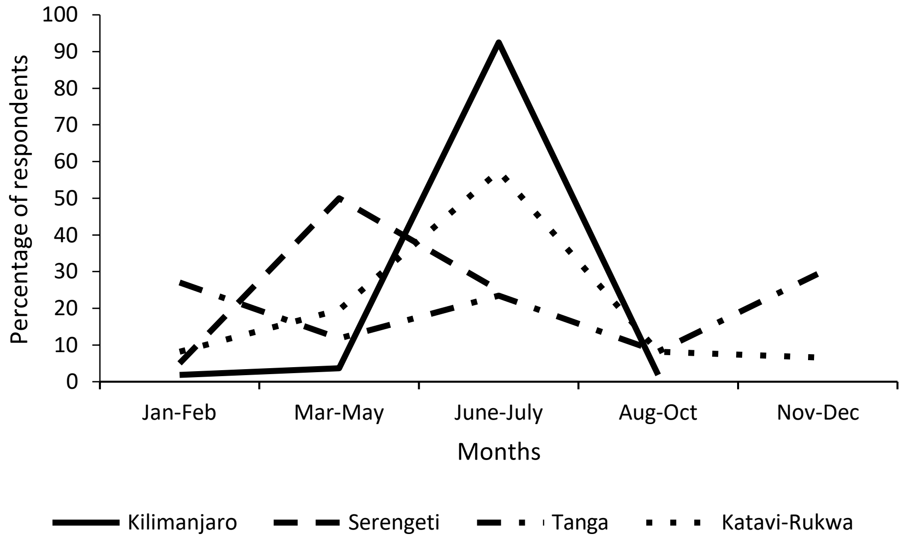

Figure 7: ***Aloe* species flowering times reported by the study respondents (in %, n=236) across the four study regions within Tanzania**

The respondents mentioned twenty *Aloe* species (n = 23) to be the most useful (Fig. 8, Appendix 3). *Aloe lateritia* Engl. *A. secundiflora* Engl. and *A. duckeri* Christian were noted to have a higher number of uses, i.e., 25, 23 and 24, respectively. The unmentioned three wild species, i.e., *A. boscawenii*, *Aloe confusa* Engl. and *Aloe fibrosa* were not known by most respondents even when a freshly collected specimen was presented to them.

The leading uses of *Aloe* species were treating malaria in humans (20%), Newcastle disease in chicken (19%), stomachache (7%), wounds, hernia, typhoid and ringworm in humans (Supplementary Data Table S2). The respondents in Rombo District had a belief that planting *Aloe* (*A. volkensii*) in a banana field will increase their yields. The *Aloe* leaves were prepared through cutting and soaking in water (48%), followed by boiling (26%) and finally squeezing out the gel (16%). Drinking of the sap (80%) was the preferred method of administering the *Aloe,* followed by direct application on the skin and wounds (17%). *Aloe* species were very effective in treating ailments permanently (97%, n = 236, with temporary relief for diabetes, toothache and numbness. A total of 171 respondents (72 %) reported no side effects during the treatment process, while a few reported diarrhoea or vomiting. Despite fewer side effects, *A. secundiflora* was reported by two respondents to have caused death when it was used in a high dose.

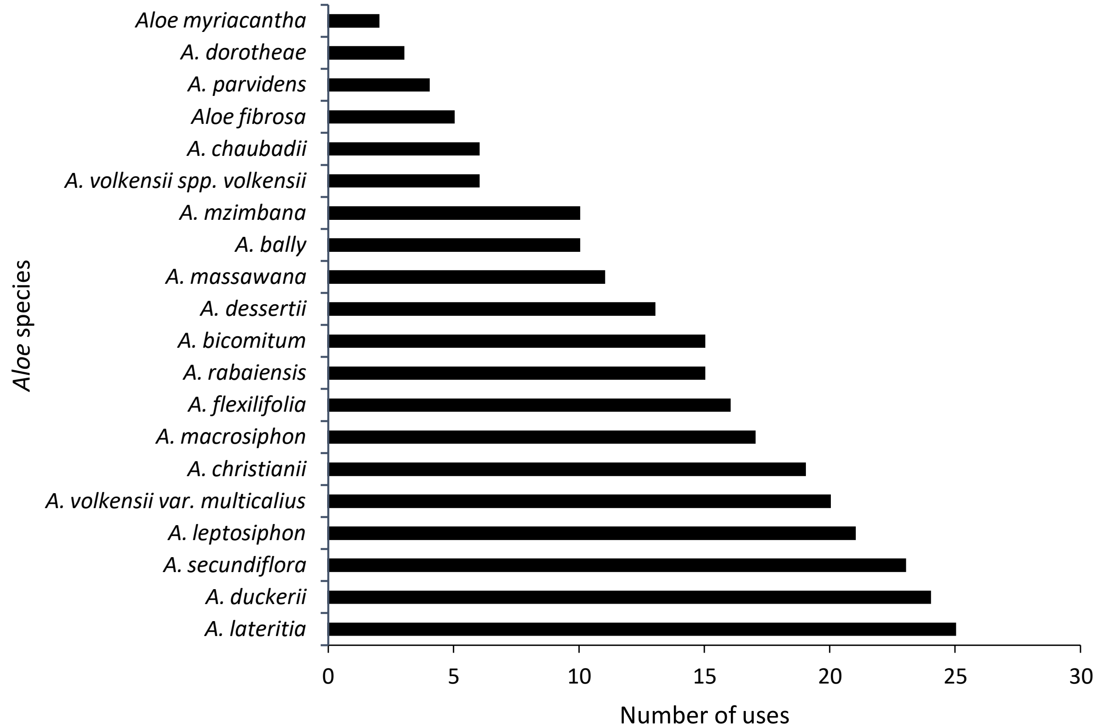

Figure 8: ***Aloe* species and their number of uses as mentioned by the 236 respondents across the four study regions within Tanzania**

4.1.4 Conservation of *Aloe* Populations in General

We found most *Aloe* species in wild populations, except for *A. massawana* (86%, N = 236), *A. volkensii* spp. *multicalius* (50%) and *A. volkensii* spp. *volkensii* (50 %), *A. lateritia* (40%) and *A. duckerii* (25%), which were mainly found in the respondents' gardens and farms. The majority of respondents (89%, N = 236) used leaves as compared with interchangeable use of leaves, stem or roots. Among those who harvested the whole plant, 72% (N = 236) used only one *Aloe* individual plant per harvest, while 28% used more than three individuals per harvesting activity. Most respondents (88%, N = 236) collected *Aloe* species from the wild while about a fifth (22 %) cultivated them in farms and home gardens. Two thirds (64%), N = 236 of respondents thought that there is no need to grow *Aloe* because proper harvesting techniques in the wild assure their availability (Fig. 9). *Aloe* species' propagation was done by planting the seedling collected from the wild by the majority (98%). Only a few respondents (2%) used the cutting of the stem to propagate.

Through the entire study area, the trade in *Aloe* species was reported to be low by only four respondents (2%) with the majority of respondents (98%) claimed that there was no trade of *Aloe* species. The latter is supported by the low prices for *Aloe* leaves that were observed in the local market: Prices ranged from 100 TSh to 1000 TSh per leaf (US$ 0.04 - 0.43).

4.1.5 *Aloe* Species Categorization, Threat and Conservation

(i) International Union for Conservation of Nature Red List Categorization of *Aloe* Species

The conservation status of 22 *Aloe* species in Tanzania was assessed, and 77% of them were found to be threatened. Figure 10 depicts the distribution of all collections and International herbarium records, while Appendix 5 contains locality maps for each species. Using the Extent of Occurrence, Area of Occupancy, and number of locations, two species were classified as Critically Endangered, ten as Endangered, five as Vulnerable, and five as Least Concern (Table 4 and Appendix 4). At high elevations (> 1500 m.a.s.l.), 40% of the *Aloe* species studied were found, while 30% were found at moderate (1000–1500 m.a.s.l.) and low (1000 m.a.s.l.) elevations. There was no significant correlation between *Aloe* species diversity and elevation ($F_{1,8} = 0.12$, $p = 0.738$, $R^2 = 0.015$). The Critically Endangered *A. boscawenii* was found at low elevation only. Of the 22 species studied, the highest species diversity was found in the Eastern Arc Mountains, followed by the Katavi-Rukwa ecosystem, Arusha, and the Coastal Forest and Serengeti ecosystem (Fig. 1). Most of the studied *Aloe* species (55%) were found in rocky areas, 74% in clumped distributions, while 26% were randomly distributed (n = 22). Most of the studied endemic *Aloe* species had higher threat categories than non-endemic species.

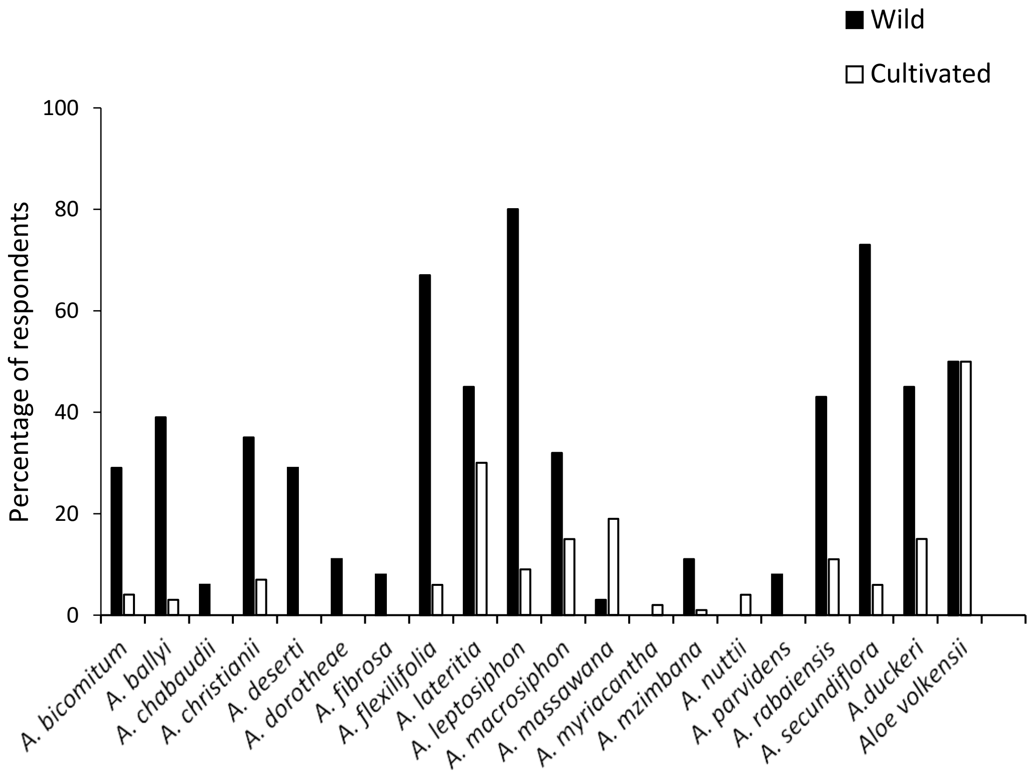

Figure 9: Responses of 236 interviewees in Tanzania across different districts on the local use of wild and cultivated *Aloe* species for herbal and/or medicinal uses across Tanzania. N =236

The 22 assessed *Aloe* species were frequently distributed in the Eastern Arc Mountains, followed by Katavi-Rukwa ecosystem, Arusha, Coastal Forest and Serengeti Ecosystem (Fig. 10). The most threatened *Aloe* species, (57%) were found in rocky areas and were in the clumped population (74%, N=23) while 26% were randomly distributed on land or along water bodies e.g *A. boscawenii, A. confusa, A. fibrosa*, *A. myriacantha*, *A. bicomitum* and *A. massawana* in some places. Most of the endemic *Aloe* species had higher threat categories than non-endemic species.

(ii) Respondents Perception on the Threat to *Aloe* Species and Conservation of *Aloe* Species

The majority of respondents (74%, N = 236) across all regions perceived *Aloe* species to be accessible or intermittently available. On the other hand, when the respondents were asked to compare the species' current availability in the last five years, 78% of all respondents in the four regions perceived the *Aloe* species to be declining. Most of the *Aloe* species were reported (based on the number of uses mentioned by respondents = N) to be found in wild populations, except for *Aloe massawana* Reynolds (86%, N = 86), *Aloe volkensii* Engl. (50%, N = 8) and *Aloe volkensii* Engl. subsp. *volkensii* (50 %, N = 92), which were mainly found in the respondents' gardens and farms. Similarly, *Aloe* leaves were reported to be a primary source of medicine (Fig. 11). In contrast, tall Aloe, particularly *Aloe ballyi* and *A. volkensii* stems were harvested and used for local beer brewing in the Kilimanjaro region. Root harvesting was reported for *Aloe deserti* A. Berger (24%, N = 29), *Aloe rabaiensis* Rendle (17%, N = 53), *Aloe mzimbana* Christian (8%, N = 29) and *A. leptosiphon* (7%, N = 87), which might have negatively affected their conservation status.

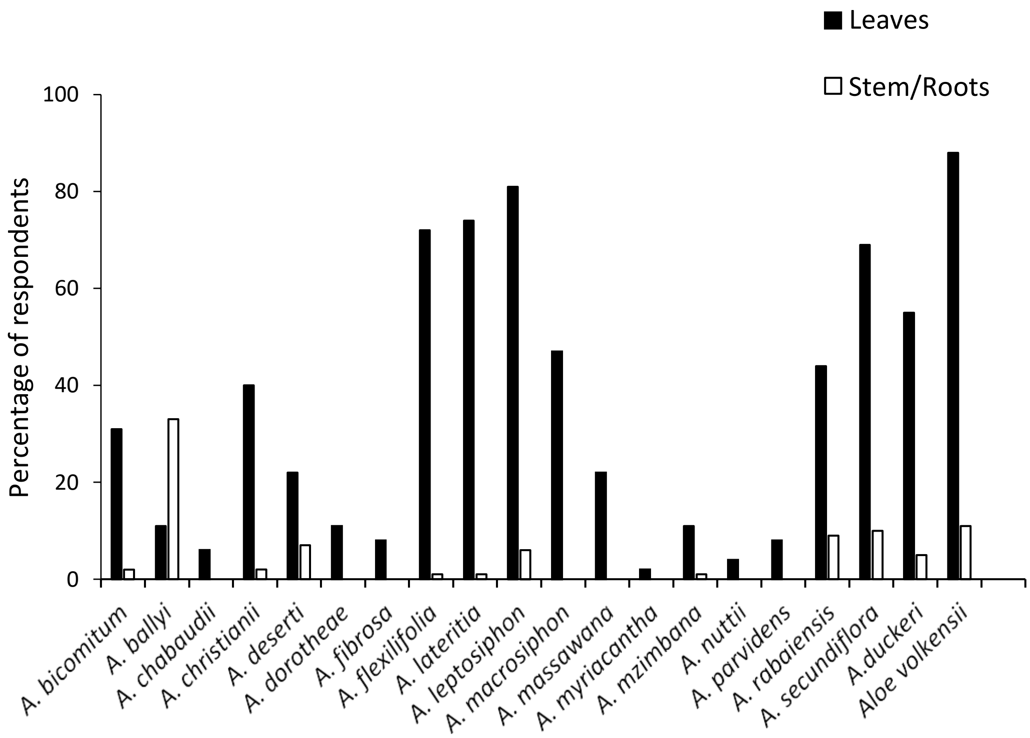

Figure 10: Preferred plant parts for utilization per *Aloe* species in Tanzania based on interviews conducted across 28 districts in Tanzania in 2018. Leaves are the most commonly used part, except for *A. ballyi,* N = 236

Table 4: The conservation status of 22 assessed *Aloe* species in Tanzania, including, their scientific names, endemism, current global IUCN status, estimated number of locations for the national assessment, and the category B1 (EOO) and B2 (AOO) in Tanzania. The description of the International Union for Conservation of Nature Red List status: CR = Critically Endangered, EN = Endangered, VU = Vulnerable, LC = Least Concern and NE=Not Evaluated

Scientific name	Endemic	Current IUCN status (all global)	Tanzania: Estimated # of locations	Tanzania: EOO (km^2)	Tanzania: AOO (km^2)	Tanzania: Newly proposed status	Threat
Aloe ballyi Reynolds	No	EN B2ab(iii) (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009a)	3	6156	32	EN B2ab(iii) (National assessment)	Overharvesting, Land for road and agriculture
Aloe bicomitum L. C. Leach	No	NE	2	6490	16	EN B2ab(iii) (National assessment)	Overharvesting
Aloe boscawenii Christian	Yes	CR D (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b)	2	43	16	CR B1b(iii)c(iii)	Restricted population and human activities
Aloe chabaudii Schönland	No	NE	3	45 607	20	EN B2ab(iii) (National assessment)	Land for agriculture
Aloe christianii Reynolds	No	NE	5	82 927	68	EN B2ab(iii) (National assessment)	Land for agriculture
Aloe confusa Engl.	No	NE	2	1265	20	EN B1ab(iv)+2ab(iv) (National assessment)	Restricted population and Floods (at Kifaru river)
Aloe deserti A. Berger	No	NT (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants 2009 g)	5	22 561	52	EN B2ab(iii) (National assessment)	Land for agriculture

Scientific name	Endemic	Current IUCN status (all global)	Tanzania				Threat
			Estimated # of locations	EOO (km^2)	AOO (km^2)	Newly proposed status	
Aloe dorotheae A. Berger	Yes	CR B2ab (v) (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009h)	7	4 109	76	VU B1ab(iii,v)+2ab(iii,v)	Fire from clearing agricultural land and grazing
Aloe duckeri Christian	No	LC (Richart, 2019a)	> 15	167 960	108	LC (National assessment)	Land for agriculture and grazing
Aloe fibrosa Lavranos & L. E. Newton	No	NE	1	65	12	CR B1ab(iv)+2ab(i) (National assessment)	Restricted population
Aloe flexilifolia Christian	Yes	CR B1ab(v) (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009i)	4	112	36	EN B2ab(iii)	Overharvest for trade products
Aloe lateritia Engl.	No	LC (Weber, 2013)	>15	624 760	320	LC (National assessment)	Fire and land for agriculture
Aloe leptosiphon A. Berger	Yes	CR B1ab(v) (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009m)	4	62	32	EN B1ab(iii)+2ab(iii)	Restricted population
Aloe macrosiphon Baker	No	NE	8	114 191	80	VU B2ab(iii) (National assessment)	Land for agriculture
Aloe massawana Reynolds	No	VU B1ab(iii)+2ab(iii) (Eastern Arc Mountains & Coastal Forests CEPF	6	13 087	112	VU B1ab(iii)+2ab(iii) (National assessment)	Urbanization

Scientific name	Endemic	Current IUCN status (all global)	Tanzania					Threat
			Estimated # of locations	EOO (km^2)	AOO (km^2)	Newly proposed status		
		Plant Assessment Project Participants, 2009n)						
Aloe mzimbana Christian	No	NE	6	26 339	40	VU B2ab(iii) (National assessment)		Pumice mining and road construction
Aloe myriacantha (Haw.) Schult. And Schult.f.	No	LC (Richart, 2019b)	10	350 683	68	LC (National assessment)		Fire from clearing agricultural land
Aloe nuttii Baker	No	LC (Richart, 2019c)	6	66 150	32	VU B2ab(iii) (National assessment)		Fire from clearing agricultural land
Aloe parvidens M. G. Gilbert and Sebsebe	No	LC (Weber & Demissew, 2013a)	2	206	12	EN B1ab(iii)+2ab(iii) (National assessment)		Land for agriculture
Aloe rabaiensis Rendle	No	LC (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009p)	4	201	28	EN B1ab+2ab (National assessment)		Land for agriculture
Aloe secundiflora Engl.	No	LC (Weber & Demissew, 2013b)	>15	215 938	216	LC (National assessment)		Overharvest for trade and Wrong harvesting method
Aloe volkensii Engl.	No	LC (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009j)	>15	174 244	152	LC (National assessment)		Overharvesting and land for agriculture

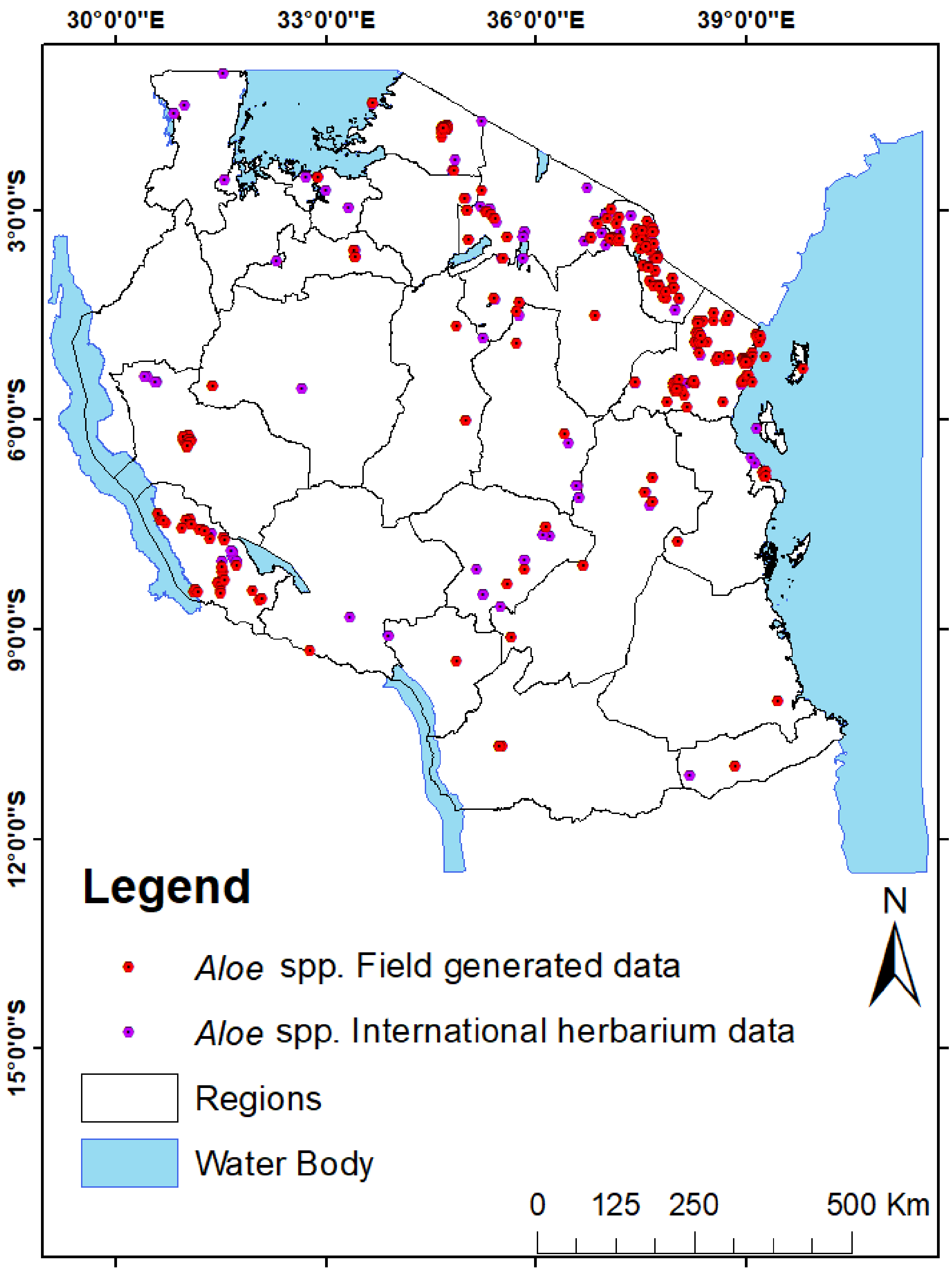

Figure 11: Distribution of the 23 *Aloe* species re-assessed in Tanzania. Red and purple dots denote field generated data respectively surveyed in 2018 and international herbarium records for the *Aloe* species' localities

According to the number of uses mentioned by respondents (N), the species with the highest EOO, i.e., *A. duckeri* (70%, N = 60), *A. myriacantha* (87%, N = 40) and *Aloe lateritia* Engl. (64%, N = 75) were perceived to be threatened (Fig. 12). Community mirth and beliefs have contributed to the conservation *A. lateritia* in Moa ward, Lushoto district. It is found in one of the sacred forests and could only be collected by Washana Clan. The *A. duckeri* was observed to be affected by agriculture and grazing activities in Nkasi district while *A. lateritia* was affected by agriculture and fire when farmers prepare the land for agriculture during rainy seasons in Lushoto district.However, the respondents reported the widespread *A. secundiflora* to be affected by overharvestingfor trade activities in the Same district by a certain company and a wrong harvesting method that resulted in most individuals' death. The three short *Aloe* in Mwanga district, i.e., *Aloe parvidens* M. G. Gilbert and Sebsebe, *A. rabaiensis* and *A. deserti* were affected by population increase and need for agriculture area, but also they were sometimes utilised for beer brewing. In contrast, *Aloe fibrosa* was not known by the majority of respondents (79%, N = 38) in Engarenairobi ward except for the few people around Simba farm area (21%, N = 38) and it was not frequently utilized compared to the more available species, i.e., *A. secundiflora, A. volkensii* subsp. *volkensii* and *A. volkensii*. Moreover, respondents perceived the *Aloe chabaudii* Schönland. (100%, N = 6) in Katavi region and *Aloe macrosiphon* Baker (65%, N = 43) in the Serengeti district to be still available.

Additionally, *A. mzimbana* (59%, N = 29), *Aloe myriacantha* (Haw.) Schult. and Schult.f. (87% = 40), *Aloe nuttii* Baker (100%, N = 10) were thought to be very rare by the respondents. *Aloe mzimbana* was affected by road construction in Kalambo district while *A. myriacantha* was affected by fire resulting from farm preparation in Nkasi district. Despite their perception, *A. mzimbana* was plentiful in Chala Hills, Nkasi district, Rukwa region. Similarly, *A. massawana* was seemingly very rare in the wild according to over three quarters (77%, N = 29) of the respondents due to urbanisation and need for agriculture land. The majority of respondents in Pongwe ward and Pangani district, Tanga region have planted *A. massawana* in their home gardens to combat its decline in the wild. Likewise, *Aloe christianii* Reynolds was also thought to be threatened by 79% of the respondents. It was observed to be affected by the increasing need for agriculture land and road construction in the Kalambo district.

Aloe boscawenii was known by only three fishermen at Boma ward, while respondents around Lake Chala did not know *A. confus*a. Farmers along Kifaru rivers mentioned *A. confusa* at the river banks in the past, but it has been washed away by frequent floods that occurred due to climate change. Although *A. flexilifolia* was alleged to be available, two respondents mentioned it was over-harvested for making commercial detergents. Furthermore, five respondents in Tanga region mentioned overharvesting of *Aloe* species such as *A. flexilifolia, A. leptosiphon, A. dorotheae*, and

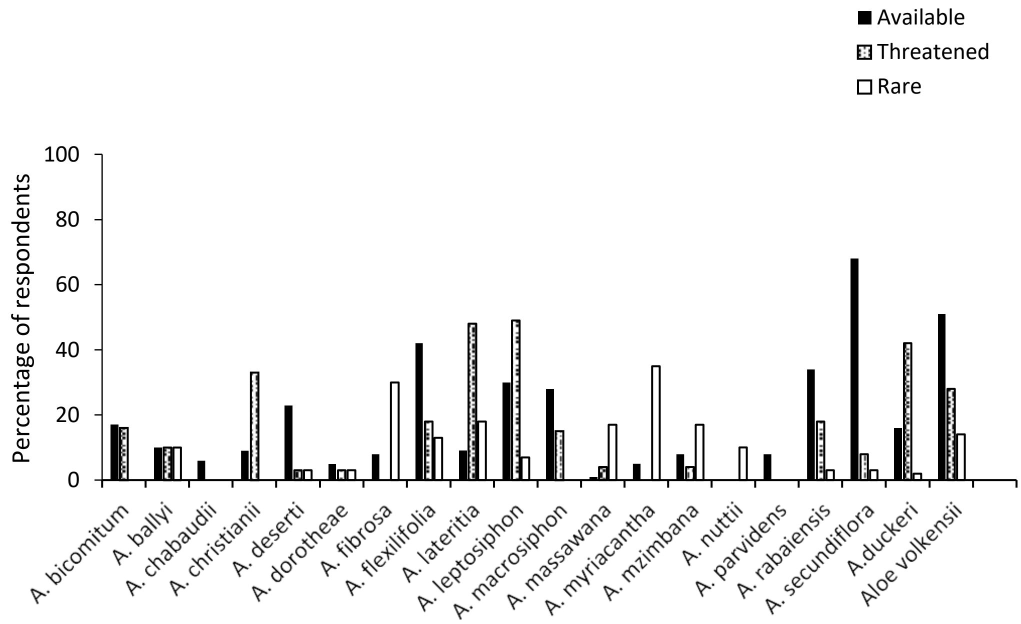

Figure 12: Local perceptions of availability and rarity of *Aloe* species in their area across 28 districts of Tanzania in the year 2018, N = 236.

The *A. lateritia*, whereby children use the gel as lubricant for their locally made bicycles *(Kikwata*, see Plate 1D). According to 43% of the respondents, overharvesting was one of *Aloe bicomitum* L. C. Leach's threats as it was over-harvested to be planted at the hotels along Lake Tanganyika. Based on the survey, human activities, including primary agriculture and the effects of climate change, are the leading causes of *Aloe* species' decline. Other threats to *Aloe* species included feeding by livestock (goats and cattle) and converting *Aloe* habitat into human settlements. For the Tall Aloe, i.e., *A. ballyi*, *Aloe volkensii* subsp. *volkensii* and *A. volkensii* were highly affected by overharvesting for beer brewing in Same district, Kilimanjaro. The unsustainable harvesting practices were detrimental to the wellbeing of wild populations of *A. ballyi* and *A. volkensii*, and may have caused their local extinction in Kisiwani and in Kideleko wards, respectively as it was reported by respondents.

Similarly, wild and domestic animals were also a threat to the *Aloe s*pecies (Plate 1). For example, the elephants were mentioned by respondents to feed and trample tall *Aloes* (*A. ballyi* and *A. volkensii*) in wards close to protected areas (e.g., Mkomazi *National Park,* Lake Chala and Kilimanjaro National Park). In the Tanga region, monkeys were reported to eat and destroy *A. lateritia*. In Katavi-Rukwa regions, livestock (cattle and goats) and beetles (*Eugaster fernandezi*) were mentioned by respondents and confirmed visually in destroying *A. duckeri* and *Aloe christianii* Reynolds. The area for settlement was quoted as causing mortality of *Aloe* species by respondents in the Serengeti district.

The perception of a general decline in *Aloe* populations was not significantly correlated with the respondent's age ($Z = -0.524$, $p = 0.600$) or the number of Aloes' uses ($p = 0.099$, $H = 5.058$). Human activities, mostly clearing land for agricultural purposes, were the leading cause of the decline in *Aloe* species availability (Fig. 13).

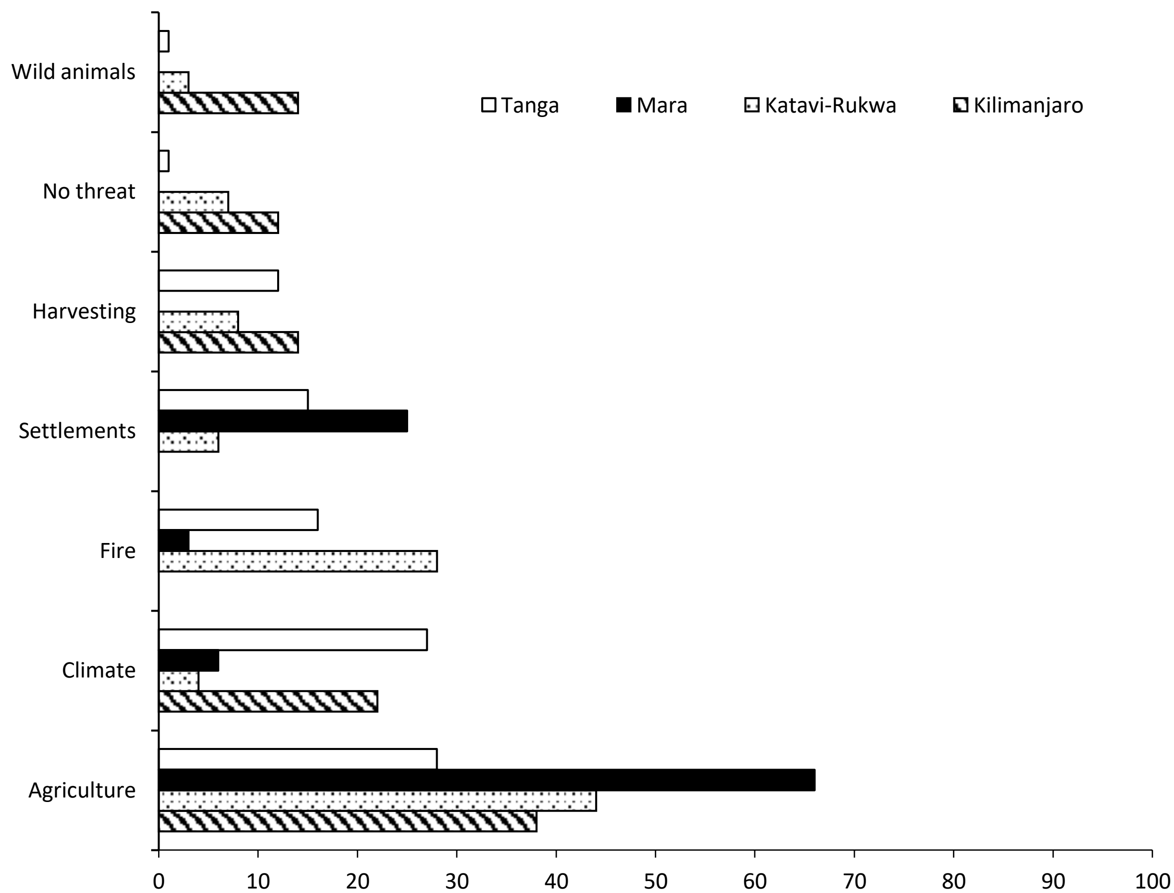

Figure 13: Factors for a potential decline of *Aloe* species based on field observations (in % of occurrence) from all four study regions within Tanzania

Plate 1: Factors affecting the availability of *Aloe* species. (A) *Aloe duckeri* after being eatenby domesticated animals, (B) *Eugaster fernandezi* enjoying the fruits of *Aloe duckerii*, (C) A young boy holding a local bicycle "Kikwata" and (D) *Aloe lateritia* under fire. Photos by Siri Abihudi

4.1.6 Propagation of *Aloe* Species

There was a significant difference in the germination of CR (*A. pembana* and *A. boscawenii*) and LC (*A. secundiflora*, *A. volkensii* and *A. lateritia*) *Aloe* seeds throughout the main effects of environmental factors and *Aloe* species (Table 5).

Table 5: Two-factorial ANOVA results to link the main effect of various treatments, *Aloe* species, and their interaction effect on seedling emergence. N = 960 seeds per *Aloe* species, n = 80 seeds per treatment

Fixed factors	df	Main effect	
		F	p
Aloe species	4	371.5	<0.0001
Temperature	2	58.5	<0.0001
Species x temperature	8	46.6	<0.0001
Aloe species	4	89.9	<0.0001
Light conditions	1	33.4	<0.0001
Species x light conditions	4	28.6	<0.0001
Aloe species	4	20.7	<0.0001
Scarification	1	15.4	<0.0001
Species x scarification	4	5.9	0.001
Aloe species	4	159.4	<0.0001
KNO_3 concentration	2	34.3	<0.0001
Species x KNO_3	8	18.5	<0.0001
Aloe species	4	50.2	<0.0001
Salinity	1	205.3	<0.0001
Species x salinity	4	5.6	0.002
Aloe species	4	22.4	<0.0001
Soil medium	1	318.7	<0.0001
Species x soil medium	4	11.7	<0.0001

Additionally, *Aloe* species and temperature treatment interacted significantly, i.e., some *Aloe* species had higher germination rates at high, some at low temperatures (Table 6, Fig. 14). The *Aloe* species germinated best at 30°C, followed by 25°C. *Aloe pembana* seeds germinated with high Final Germination Percentage (FGP) in all the temperature treatments and highly preferred moderate and higher temperatures for germination. The FGP of *A. pembana* was 81%, twice as high as that for *A. lateritia* (31%) at 35°C and three times high as that for *A. secundiflora* at 30°C. *Aloe lateritia* and *A. boscawenii* performed better at moderate temperatures of 25°C and 30°C, respectively. *Aloe secundiflora* had a low germination percentage (20%) although germinated better at the higher (29%) and lower temperature regimes compared to the moderate temperature of 30°C (13%). Surprisingly, *A. volkensii* did not germinate at any temperature. The CR *A. boscawenii* had no significant difference with the LC *A. volkensii* and *A. lateritia* at 35°C. Although *A. pembana* germinated well in high temperature, it outshined other *Aloe* species at low temperatures of 25°C.

The constant-dark conditions had a significantly higher FGP than light conditions, except for *A. boscawenii*, which did not germinate at all (Fig. 14). Both *A. secundiflora* and *A. lateritia* had the germination rate of 86% for the dark treatments, which was almost twofold as high as that of *A. pembana* (45%). There was no significant difference between *A. pembana*, *A. boscawenii* and *A. volkensii*, nor between *A. pembana* and *A. secundiflora.*

The majority of *Aloe* species performing better when not scarified and their interaction with scarified seeds was significant between species. Un-scarified *Aloe* seeds had a slightly, but not significantly, higher germination percentage than scarified seeds (Table 6). *Aloe secundiflora* which had a tiny slighter seed, failed to germinate after scarification but grown well (51%) when un-scarified.

Furthermore, *Aloe* seeds' germination percentage differed significantly across KNO_3 concentrations (Fig. 14) with high (0.1 mg/L) KNO_3 concentrations not favouring germination. In contrast, low concentrations (0.01 mg/L) did favour all the *Aloe* species except for *A. boscawenii.* There was no significant difference between the control (0 mg/L KNO_3) and 0.01 mg/L KNO_3 (Fig. 14). Moreover, the difference between the *Aloe* species that germinated well (*A. pembana* and *A. lateritia*) under high concentration of 0.1 mg/L KNO_3 and those that did not perform well (*A. boscawenii*, *A. secundiflora* and *A. lateritia*) was not significant. The FGP of *A. pembana* (76%) was twice as high compared to *A. secundiflora* (35%) and *A. volkensii* (35%). *Aloe boscawenii* differed significantly from other *Aloe* species at 0.01 mg/L KNO_3 whereas; there was no significant difference between *A. pembana* and *A. lateritia* nor between *A. secundiflora* and *A. volkensii.*

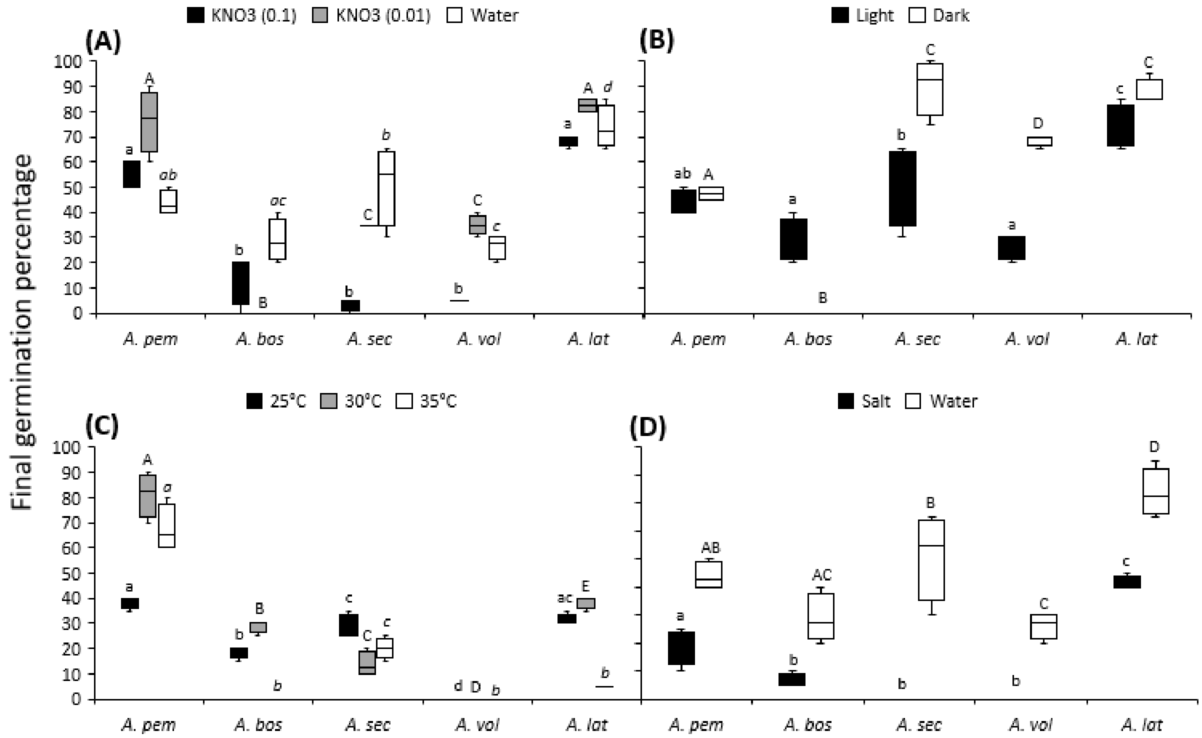

Figure 14: Final germination percentage of *Aloe* seeds grown at different (A) temperatures, (B) light condition, (C) potassium nitrate concentration and (D) salinity. Different letters (in the capital, in small and in italics) indicate significant differences across germination percentage means of different species at specific treatment according to Turkey's post hoc tests (p < 0.05). Different colours indicate different treatment levels. The two CR species are *A.pem* and *A. bos* while the LC species are *A. sec, A. vol* and *A. lat*. *A. pem = Aloepembana, A. bos = Aloe boscawenii, A. sec = Aloe secundiflora, A. vol = Aloe volkensii, and A. lat = Aloe lateritia*

Application of saline water as a growth medium did not result in germination within the first three weeks. Nevertheless, seeds germinated right away when the saline water was added into distilled water medium, except for *A. volkensii* and *A. secundiflora* (Fig. 14, Table 6). *Aloe lateritia* seeds germinated magnificently (41%), followed by *A. pembana* (19%), and *A. boscawenii* (6%) when recovered from saline water. The seeds grown with distilled water medium surpassed those recovered from saline water for all *Aloe* species tested (Fig. 14). The initiation of the germination process was faster (2.6 days) (t = 12.5, p = 0.003) for the seeds recovered from saline water compared to the seeds on distilled water medium (5.6 days).

There was a slight but not significant tendency for most species to grow better without soil than on filter paper water medium, except for *A. volkensii* (t = 5.66, p = 0.001), which achieved better with soil medium than in filter paper water medium (Table 6). The Tukey indicates no significant difference among the CR species (*A. pembana* and *A. boscawenii*) with the two LC species (*A. secundiflora* and *A. lateritia*) although the later had a significant difference.

4.1.7 In vitro Culture of *Aloe* Species

(i) Culture Initiation, Proliferation, and Multiplication

Generally, contamination of the cultured samples (N = 349) occurred within the 5^{th} and the 10^{th} day after culture initiation. Contaminations were mainly due to fungal infections (66%, N = 230) at all concentration levels of NaOCl and $Hg(NO_3)_2$ tested. Among the concentration tested, NaOCl (6%) both treated and untreated with an antifungal agent (Metalaxyl 8% + Mancozeb 64%) had the highest proliferation percentage (19%) compared to other concentrations (Table 7). This antifungal agent's application caused the fungus to be attached to the explant sample, and the media was free from contamination, which shows the contamination was from an endophytic fungus and not from the medium (Plate 2).

Application of $Hg(NO_3)_2$ (0.1%) reduced the contamination (24%) but ended up drying the cultured samples, likely due to the high concentration (10%) of NaOCl (48%). The contaminated materials that proliferated were treated by disinfecting them with 6% NaOCl for 1 min before subculturing. Surprisingly, there was no contamination in the sub-cultured materials as it was the case in the initiation stage. Seeds proliferated best (22%, n = 59) followed by meristem (17%, n = 58) shoot tips (9%, n = 58) and roots (8%, n = 58) (Fig. 15). In contrast, leaf tips and sheaths did not germinate at all. Although the seeds had the highest proliferation percentage, they ended up drying and never developed lateral shoots. The proliferation occurred within 6-10 days after in-vitro culture initiation.

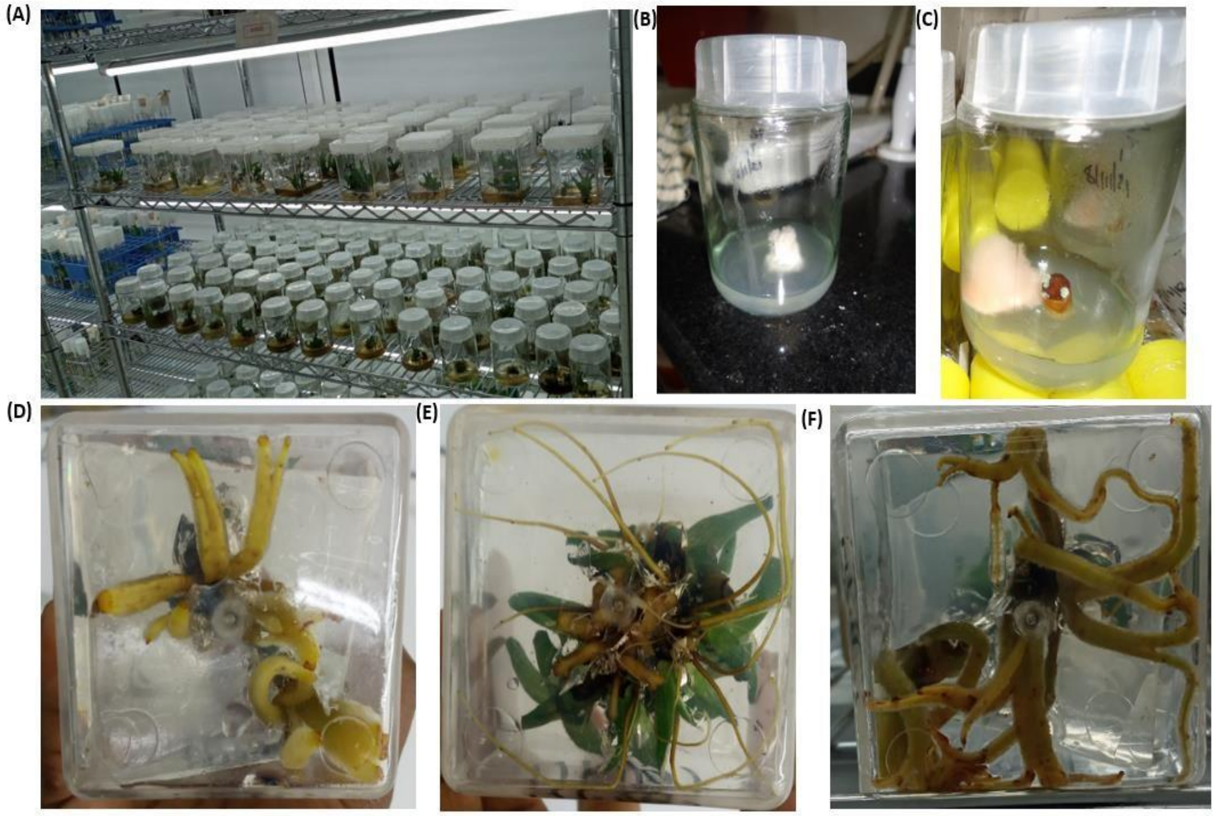

Plate 2: The initiation of proliferation process (A), contamination-free medium after the antifungal agent (B), contaminated medium in un treated medium, rootmorphology when NAA (1 mg/L) (D), IBA (1 mg/L) and combination of NAA and IBA (2:1) (F) were used during rooting

Table 6: Effect of different concentrations of NaOCl and $Hg(NO_3)_2$ in the prevention of contamination and its effect on the proliferation and germination of cultured samples. Numbers behind NaOCl indicate concentration (N = 349)

Concentration %	Etha nol	Cultured samples (n)	Contamina ted (%)	Proliferat ed (%)	Not germinated (%)
NaOCl (3)	70	55	100	0	0
NaOCl (4)	70	42	71	10	19
NaOCl (5)	70	42	74	12	14
NaOCl (6)	70	42	57	19	24
NaOCl (7)	70	42	71	14	14
NaOCl (10)	96	42	48	0	52
NaOCl (6) + (Metalaxyl 8% + Mancozeb 64%)	70	42	71	19	10
$Hg(NO_3)_2$ (0.1%)	70	42	24	5	71
Total		**349**	**65**	**10**	**26**

Browning was another challenge faced during the proliferation and multiplication stage but was never observed during the rooting phase. In the first culture, the formation of lateral shoots ranged from 2 to 4 number of new shoots, but in the subsequent cultures, the number of shoots increased to 4 to 6 per cultured sample for every six weeks. The lateral shoots increased with an increase in the number of subcultures in the new medium. A total of eight subcultures were performed during this study.

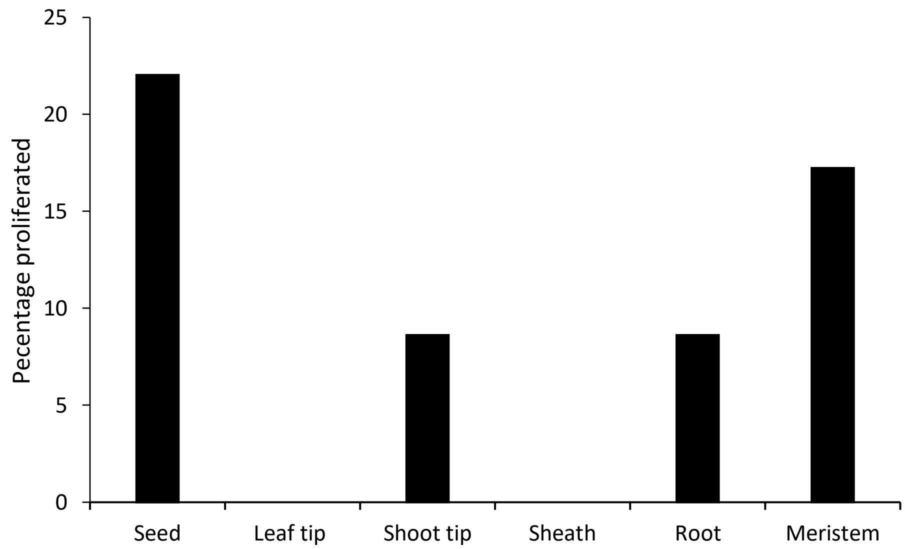

Figure 15: Percentage of *Aloe boscawenii* plant parts that proliferated after culture initiation. N = 349 ex-plant materials

(ii) Rooting and Acclimatization

Rooting

There was a significant difference on the root length (*df* = 6, *F* = 4.22, *p* = 0.00053), the number of roots (*df* = 6, *F* = 6.41, *p* < 0.0001) and shoots (*p* = 0.0012, *df* = 6, *F* = 4.03) produced by α-Naphthalene acetic acid (NAA) and Indole-3-butyric acid (IBA) and their combination (Fig. 16). When IBA was in 1 mg/l concentration, it gave very long roots (4.33 cm) followed by the combination of NAA and IBA in a 2:1 ratio (3.99 cm). Moreover, NAA (1 mg/L and 2 mg/L) gave health plantlets and huge roots followed by the combination of NAA and IBA (2:1). On the other hand, the plantlets rooted with IBA (1 mg/L and 2 mg/L) gave very slender roots. The number of roots produced was significantly favored by 1 mg/l NAA (27.2 roots) followed by 2 mg/L NAA (15.8 roots). The number of proliferated shoots produced during rooting was mostly favored by 2 mg/L of IBA (3.5) followed by 1 mg/L of NAA (3.3). The number of roots produced was higher in the 2 mg/L of NAA than in other concentrations.

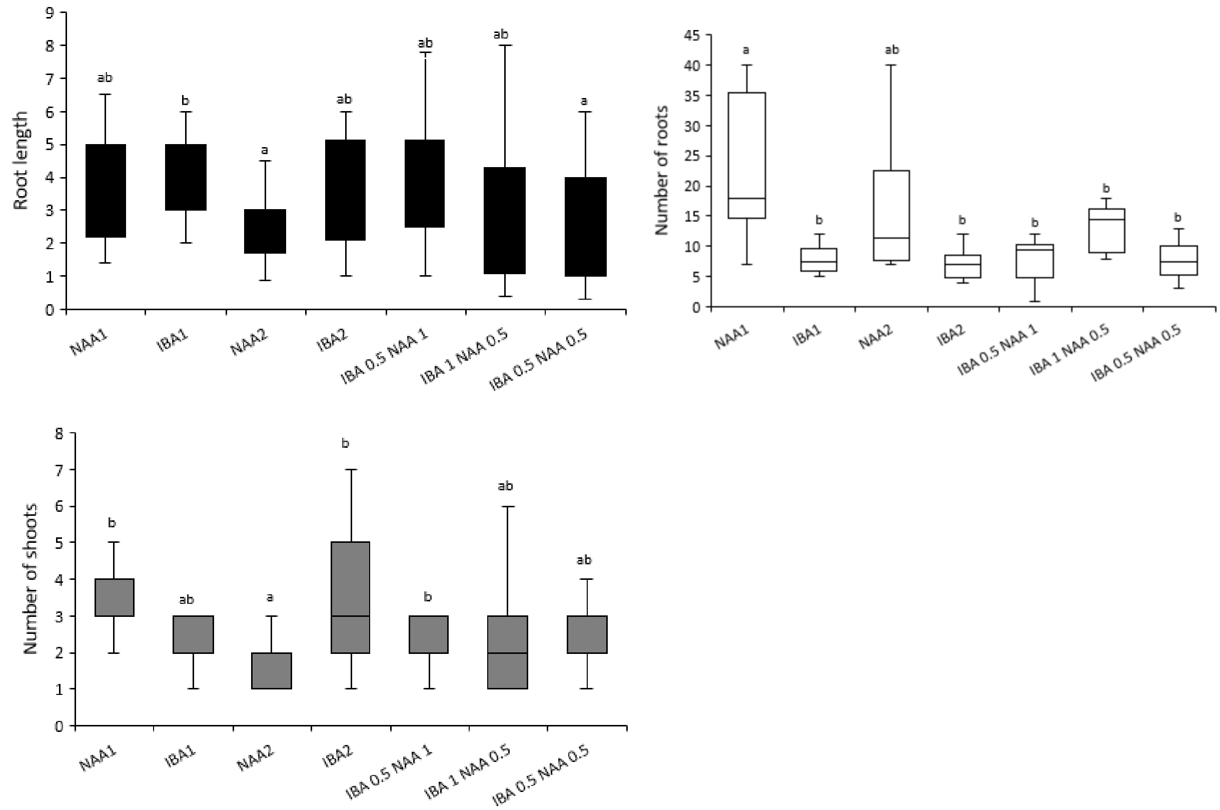

Figure 16: Root length, number of roots and shoots of *Aloe bocawenii* plantlets produced by different concentration of NAA, IBA and their combination

When the moderate amount of gellan gum was used 1 mg/L in (20 mL), the rooting of plantlets was successful with little mortality (6%) for NAA and IBA in the ratio of 1:2, and 2:1 and no mortality was observed in 1:1 proportion. On the other hand, when the gellan gum was in excess (2 gm/L) and half (10 mL), the rooting of 1 mg/L NAA (13%) was affected, with a 44% mortality rate as compared to the 1 mg/L IBA in which the mortality was 25%. The 20 mL of 1 gm/L for both NAA and IBA did not result in any mortality of the cultured plant.

Acclimatization

The majority of hardened plantlets (74%, n = 90) survived with no significant difference ($F = 0.64$, df = 6, $p = 0.674$) on how different hardening media supported the acclimatization of the plantlets (Plate 3). The multiplication of the plantlets during hardening was highly favored by the combination of Sand + Sawdust + Coco peat media, which was significantly different from other hardening media (Table 8). The plantlets grown on sand media were green, healthy and resembled the mother plant in the wild. It was followed by the Sand + Coco peat media and Sand + Sawdust + Coco peat. On the other hand, the plantlets grown on sawdust were yellowish and unhealthy, followed by the Sand + Sawdust medium. When the three mixtures (Sand + Sawdust + Coco peat) were repeated, the acclimatization rate was high (90%).

Table 7: One-way ANOVA testing which soil medium was best for plantlets to survive on average per each soil medium (±SE) during the acclimatization process in the screen house. Different letters across rows represent significant differences according to Tukey's Post Hoc tests ($p < 0.05$)

Soil Medium	No. of plantlets survived	Multiplication of plantlets
Sand	4.0 ± 0.6a	12.0 ± 1.7 a
Sawdust	3.0 ± 0.6a	8.3 ± 1.5a
Coco peat	3.3 ± 0.3a	8.7 ± 0.7a
Sand + Coco peat	3.7 ± 0.7a	13.3 ± 2.3a
Sand + Sawdust	3.7 ± 0.3a	7.7 ± 0.3ab
Sand + Sawdust + Coco peat	4.7 ± 0.3a	18.7 ± 1.3b
F	0.64	2.16
P	0.674	0.127

Plate 3: Different acclimatization media such as Sand, Sawdust, Coco peat and their mixture used during the hardening of *Aloe boscawenii* after tissue culture in 2020 at NM-AIST screenhouse, Photos by Siri Abihudi

4.1.8 Comparison Between *A. boscawenii* Establishment in Cuttings and Seeds

The shoots sprouting rate of cuttings was hypothesized to be greater than the sprouting level of seeds. This difference was significant at t (6) = 2.38, df = 6, p = 0.027) (1 tail). Although the germination level was higher in cuttings, shoot sprouting initiation took a shorter time in seeds (5th day) than in cuttings (11th day).

4.1.9 Soil Parameters that Favor the Growth of *Aloe boscawenii*

There was a significant difference in Sulphur and Zinc concentration between areas with *Aloe* and those with no *Aloe* (Table 9); hence Sulphur and Zinc are essential elements for the growth of *A. boscawenii* species.

Table 8: T- test of the Mean (± SE) Soil chemical properties in areas where *A. boscawenii*was observed (present) and at a distance of 6 m (20 feet's) away from where it was not observed (absent)

Soil properties	present	absent	*T*	df	*p*
OC (%)	1.2±0.5	1.2±0.6	0.0	2	0.974
Ph	5.4±0.1	5.7±0.3	-1.8	2	0.214
EC (ds/cm)	0.9±0.1	1.0±0.1	-0.8	2	0.507
TN (%)	0.1±0.0	0.1±0.0	-0.2	2	0.850
P (mg/kg)	4.0±1.2	4.5±0.9	-1.8	2	0.220
Ca (cmol(+)/ kg)	1.4±0.7	2.1±1.6	-0.3	2	0.767
K (cmol(+)/ kg)	0.0±0.0	0.1±0.0	-0.7	2	0.577
Mg (cmol(+)/ kg)	0.1±0.1	0.1±0.0	-0.3	2	0.768
Na (cmol(+)/ kg)	0.1±0.0	1.2±1.2	-0.9	2	0.453
S (mg/kg)	40.3±6.8	67.3±8.3	-4.9	2	0.038*
Mn (mg/kg)	9.1±4.0	7.0±1.0	0.6	2	0.623
Al (mg/kg)	278.5±75.6	101.1±21.3	1.9	2	0.203
Zn (mg/kg)	0.7±0.2	0.5±0.1	4.5	2	0.046*
Fe (mg/kg)	91.7±13.8	62.2±8.2	1.4	2	0.289
B (mg/kg)]	0.0±0.0	0.0±0.0	-0.1	2	0.939

* Stands for significant at $p < 0.05$

4.2 Discussion

4.2.1 Ethnobotanical Knowledge of *Aloe* Species in Tanzania

Generally, the genus *Aloe* showed a positive effect on human wellbeing in the study area, given its multiple uses ranging from medicinal use, local beer brewing to acting as an ornamental plant. The use of *Aloe* species has been recognized worldwide (Bjorå *et al*., 2015; Grace *et al*.. 2008; Manvitha & Bidya, 2014) and Tanzania can, therefore, use its outstanding diversity of *Aloe* species to benefit its people if the utilization can be done sustainably.

4.2.2 Folk Taxonomy of *Aloe* Species

Most respondents from the study regions were able to distinguish the *Aloe* species that were locally present. The locals were also able to differentiate *Aloe* species from the morphological similar genus *Agave* based on spines along the leaf edges in *Aloes* and their absence in *Agave* species, e.g., *Agave sisalana* Perrine ex Engelm. Confusing these two genera dates back to at least the 17th century when *Agave* was considered part of the *Aloe* family (Bogler *et al.,* 2005). In this study, folk taxonomy used the same principle applied in scientific binomial nomenclature (Berlin, 1973), although names were more firmly based on prominent vegetative structures. In this case, the primary name (*Kithapa* = *Aloe*) included all species in the genus and secondary names (*kietha* or *kidori* = tall or short, respectively) often identified a species or at least similar species. Similar naming systems have been reported in studies of other species and in different geographic areas (Berlin, 1973; Berlin *et al.,* 1966), who also hypothesized that folk taxonomy is universal among cultures. In this study, folk taxonomy knowledge is possibly in decline since most people particularly young ones referred to *Aloe* species as '*Aloe vera*' rather than using local names. In this case, the decline of knowledge of folk taxonomy might be due to *Aloe vera* products' global importance. This now represents the "common name" for any *Aloe* species encountered (Otieno *et al.,* 2015). Furthermore, urbanization, the spread of medical facilities and a formal education system may be contributing to decreasing local knowledge (Amir *et al.,* 2019; Srithi *et al.,* 2009) and might replace the use of folk taxonomy. There is a trend of fewer young people being exposed to *Aloe* species in their natural habitats and few young Tanzanians accompany those with knowledge of folk taxonomy (i.e., grandfathers, fathers and, sometimes, mothers) when they are collecting or working in areas with native plants (Sõukand & Kalle, 2011).

4.2.3 General Uses of *Aloe* Species

In this study, traditional knowledge was more common in older respondents. This highlights the high risk that the knowledge might vanish, as it was previously (Amir *et al*., 2019; Srithi *et al*., 2009). Further, men were more involved in the transfer of ethnobotanical knowledge and collecting of Aloes in the wild than women. This might reflect the traditional role men play in the collection of herbal medicines from the wild (Cunningham, 1993; Torres-Avilez *et al.,* 2016; Van Hoang *et al.,* 2008).

The two *Aloe* species (*A. lateritia* and *A. secundiflora*) most commonly used by the respondents were widely distributed in Kilimanjaro, Mara and Tanga regions. Similarly, *A. duckeri* was found in most parts of Katavi-Rukwa regions, where it was commonly used in traditional medicine. Although *A. secundiflora* had yellow exudates, which were thought to contain healing chemicals (Reynolds, 2004b), it had fewer uses than *A. lateritia* and *A. duckeri*, which both had colorless exudates. These three commonly used *Aloe* species are a succulent rather than a grassy growth form, and other studies showed that this *Aloe* form is the preferred one (Bjorå *et al*., 2015; Grace *et al*., 2015). In this study, *Aloe* species were most commonly used in traditional human and veterinary medicine, but some distinctions depend on the region. For instance, in the Tanga region,use was influenced by the taste, i.e., *Aloe volkensii* was referred to as "true *Aloe*" and was preferredfor medicinal purposes because of its bitter taste. Similarly, in Kenya, *A. lateritia* is not preferred for therapeutic use due to its lack of a bitter taste (Bjorå *et al.,* 2015). The tall Aloes' stems were an essential ingredient in beer brewing, and must had some medicinal effects. For example, *Aloe vera* wine was found to have antibacterial effects on pathogenic bacteria including *Salmonella typhimurium, Staphylococcus aureus* and *Escherichia coli* (Trivedi *et al.,* 2012). Many *Aloe* species in this study were used for similar purposes as in other East Africa areas (Table 10; Amir *et al.,* 2019; Bjorå *et al.,* 2015; Grace, 2011; Wabuyele, 2006). The usage of *A. mzimbana, A. volkensii* spp. *volkensii, A. leptosiphon, A. parvidens* and *A. bicomitum* is reported in this study for the first time in East Africa (Table 3 and Supplementary Data Table S2). Not a single *Aloe* species was mentioned to occur in all five studies reported in Table 10, which might be related to *Aloe* species' strong endemism (Wabuyele *et al,.* 2006; Reynolds, 2004).

Most respondents indicated that they use *Aloe* species based on their morphological structures, although locals mentioned that it is difficult to distinguish poisonous from non-poisonous species. For example, *A. ballyi,* a poisonous species (Reynolds, 2004), looks similar to *A. volkensii,* a non-poisonous and commonly used species. Thus, confusing of these two species may threaten the health of consumers. The misidentification of plant species either from the same family or different families have been reported to result in kidney injury (Colson & De Broe, 2005) and sometimes even death (Colombo *et al.,* 2009; Furer *et al.,* 2011; Ndhlala *et al.,* 2013). Furthermore, an overdose of some medicinal *Aloe* species can result in electrolyte imbalances, low blood sugar levels and even death (Surjushe *et al.,* 2008).

This study showed that *Aloe* species' use has not yet led to a decline in population sizes except for the highly uprooted *A. ballyi* and *A. volkensii*. These results may stem from respondents' behavior as most people (89%) preferred to harvest only a few leaves rather than stems and roots for treating diseases. A lack of a decline has also been observed in other studies of *Aloe* (Amir, 2019; Kidane *et al.,* 2014). An exception to stable populations may be for *A. ballyi, A. volkensii*, *A. deserti*, *A. rabaensis* and *A. parvidens* as they were uprooted for making local beer and *A. leptosiphon* for medicinal purposes. If these species' populations continue to decline, it may be necessary to limit and control the harvest of plant parts. Respondents and the observations also showed that some species, like the endemic *A. boscawenii,* are not frequently used, but they are often uprooted, burned or destroyed. This species and other endemics, which are similarly at risk of extinction, should be given high conservation priority. The majority of respondents (81%) were able to access *Aloe* species within a radius of up to 1 km from their homes, suggesting that most common *Aloe species* are still readily available. A plant species is considered to be abundant if a collector has to move only a short distance to find an individual of that species (Castle *et al.,* 2014). However, the clearing of areas with a high abundance of medicinal plants for development purposes has been found to increase the distance and time a collector devotes to searching for a particular plant (Castle *et al*., 2014; Magoro *et al*., 2010).

Table 9: Different uses of *Aloe* species recorded in this study (√) and according to five other studies (a-e) in East Africa

	Human ailments																	Animal ailments			
Scientific name	Urology	Skeleto-muscular	Respiratory	Poisons	Parasites/metazoan	Nervous system	Metabolic issue	Infections	Gastrointestinal	Gynecology	Ophthalmic	Food	Dermatologic	Cardiovascular	Cancer	Antidote	Others	Chicken (Newcastle)	Cows	Others	Non-medicine
A. ballyi		√	√					√	√			√		√			√, d				
A. bicomitum			√					√	√					√		√		√			
A. chabaudii						√		√	√, e, b	e	b			√		√	b	√			
A. christianii		√	√		√	√		√	√	e, b			e	√, e		√		√			
A. deserti	√	d, e	d, e			√	d, e	√, d,e	√			√	√	√				d, e	√	d, e	d
A. dorotheae							√	√					√					√			
A. duckerii		√	√	√	√	√		√	√	√			√	√, b		√		√			
A. flexilifolia						√	√		√	√				√	e		√	√			
A. lateritia		√	√, d, e	b	√, d, e	√, d, e	√	√, d, e	√d	√	d, e	d	√, d, e	b, e		d	√, b	√, d, e	b	d, e	d, b
A. leptosiphon		√	√	√		√	√	√	√				√	√				√	√		
A. macrosiphon			√		√	√		√	√, e	√			√	√			c				
A. massawana					√	√	b		√					√				√			
A. mzimbana					√			√	√	√			√	√				√			
A. parvidens		√						√						√		√					
A. rabaiensis	√	√	√	b		√		√	√			√	√c	√					√		
A. secundiflora	√, d, e	√, d, e	√, b, d, e	b	e	√, b, d, e	√, d, e	√, d, e	√, b, d, e		√, d, e	√, a, d	√, b, d, e	√, b, d, e			√	√, b, d, e	√, d	b, d	b, c, d
A. volkensii subsp. *multicalius*		√	√, b, d, e	b		√	√, e	√	√		√, e	√, b	e	√, e			√	√	√, d	d	d
A. volkensii subsp. *volkensii*							√	√	√					√				√	√		
Aloe sp. (SA 45)						√		√	√									√			
Aloe sp. (SA 53)						√		√	√					√				√			
Aloe sp. (SA 87)						√												√			

The diseases were categorized based on the classification of diseases and remedies in ethnomedicine and ethnopharmacology (Staub *et al*., 2015). The best time for collecting flowering voucher specimens of Aloes in Tanzania was immediately after any of the two rainy seasons in the regions (February to April) as it was reported by Van Wyk and Smith (1996) and June to July, respectively. This finding corresponds with that of other studies, which indicated that flowering in most *Aloe* species is linked to climatic patterns (Forbes *et al.,* 2009; Symes, 2017).

4.2.4 *Aloe* distribution and Conservation

Aloe species in this were widely distributed across Tanzania, and many species were still locally available and well known by local people. However, there has been little domestication of *Aloe* species. Conserving the species by cultivating them *ex-situ* reduces pressure on the wild populations (Cocroft, 2005; Schippmann *et al.*, 2005). However, in a study on ethnoveterinary medicinal plants in the North West Province of South Africa (van der Merwe *et al.,* 2001), traditional healers claimed that cultivated medicinal plants were less effective in treating ailments compared to wild counterparts. This can be explained by the high concentration of secondary metabolites produced by wild plants as a defense against diseases, herbivores, and harsh environmental conditions (Dubey, 2011). This opinion could potentially prevent reducing pressure on wild populations. Additionally, overharvesting might be the main cause for the local extinction of *A. volkensii*, as observed in the Kideleko ward. Fire's effect has also been more harmful to the *Aloe* seedlings and juveniles, while the mature individuals are fire-tolerant (Cousins & Witkowski, 2012).

Furthermore, local extinction of preferred *Aloe* species might cause people to shift their preference to other species, further impacting Aloes' conservation in the area. The general decline of *Aloe* populations in the wild mentioned by the respondents highlights the need for conservation actions to be taken to ease the pressure on wild populations. Domestic and wild animals such as goats, cattle, elephants, and beetles were mentioned by respondents and observed to destroy *Aloe* species and their wild habitat (Plate 1). This has been previously observed by Bhaludra (2013) and Breebaart *et al.* (2002). Elephants (Cousins *et al.*, 2013; Wiseman, 2001) have similarly been reported to contribute to high levels of destruction of Aloes in South Africa. However, in addition to damaging plants, some plant-insect interactions are essential in the life cycle of Aloes. For instance, insects can also be crucial for pollination; *A. plicatilis* (L.) Mill. is pollinated by insects such as honeybees (*Apis mellifera* L.) and monkey beetles (*Scelophys trimeni*) (Péringuey, 1902; Cousins *et al.*, 2013).

4.2.5 Red List Assessment and Re-assessment of 23 *Aloe* species in Tanzania

(i) Natural Habitats of Threatened *Aloe* Species

In this study, 22 *Aloe* species present in Tanzania were reassessed using EOO and AOO criteria to determine their threat level. There was an upgrade of 18% studied *Aloe* species (*A. deserti*, *A. nuttii*, *A. parvidens*, and *A. rabaiensis*), downgrade of 14% (*A. dorotheae*, *A. flexilifolia*, and *A. leptosiphon*), while 39% species (*A. ballyi*, *A. boscawenii*, *A. duckeri*, *A. lateritia*, *A. massawana*, *A*. myriacantha, *A. secundiflora*, and *A. volkensii*) retained their current IUCN Red List status in Tanzania. The remaining species were assessed for the first time (*A. bicomitum*, *A. chabaudii*, *A. christianii*, *A. confusa*, *A. fibrosa*, *A. macrosiphon*, and *A. mzimbana*). It is important to note that EOO calculations mostly downgrade the threat status if the AOO and locations are not considered. This had previously been done for the *Aloe* species in Kenya (Wabuyele *et al*., 2006). The inclusion of the AOO and number of locations into the analysis gives a more accurate representation of highly distributed species (Solano & Feria, 2007), since the AOO analysis also takes the physically occupied area into account.

All four previously assessed Critically Endangered *Aloe* species remained threatened; one retained its Critically Endangered status (*A. boscawenii*), two were categorised as Endangered (*A. flexilifolia* and *A. leptosiphon*), and one as Vulnerable (*A. dorotheae*). This is not surprising since rare and endemic species are often at a competitive disadvantage, compared to Least Concern species, when competing for space (Murray *et al*., 2002). *Aloe confusa*, which was re-assessed as an Endangered species in Tanzania, was found in only two locations along Lake Chala that could not be reached by the local community. During the survey, *A. boscawenii*, which had last been observed in 1953 (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b), was re-discovered in the coastal area of the Boma ward in Tanga region. *Aloe boscawenii* had four sub-populations along the coasts of Boma, Manza, and Moa, with two of them in Manza being adjacent to commercially active salt mines, posing a hazard if more ponds are built, while the two sub-populations in Moa and Boma were adjacent to the ocean, hence vulnerable to flooding. As a result, sub-populations in Boma and Moa were considered as a single location and the two in Manza were considered to be a single location, hence we consider two locations for *A. boscawenii*. One villager stated that *A. boscawenii* was available along the coast of Mombasa, Kenya, as previously reported (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b). Five locations were identified for *A. flexilifolia* and *A. leptosiphon*. The previous IUCN Red List assessment only identified two locations for both species. As it will be discussed later, the Critically Endangered (*A. boscawenii*) and Least Concern (*A. lateritia*, *A. secundiflora*, and *A.*

volkensii) *Aloe* species germination responded to different environmental parameters according to their IUCN Red List status.

The assessment resulted in a downgrade for most *Aloe* species that had previously been categorised as Critically Endangered. For example, the Critically Endangered *A. flexilifolia* and *A. leptosiphon* were assessed as Endangered and *A. dorotheae* as Vulnerable. The downgrade of these species was due to the identification of new locations that had not been reported in the previous assessment. *Aloe dorotheae* was found in previously undocumented areas beyond the Handeni district, resulting in an increased EOO of 4109 km^2.

It is important to note that a species' rarity does not necessarily mean it is highly endangered if human activities and natural processes do not negatively impact its natural population levels (Oredsson, 1997). In this study, the existence of the Endangered *A. confusa* was not known to the people living around Lake Chala in Rombo district, Kilimanjaro region. Such unawareness might help its conservation in the wild. That unawareness can benefit conservation as has been suggested in previous studies. For instance, Oredsson (1997) argues that the presence of a rare plant species in two poorly visited localities in Sweden helped maintain its population.

On the other hand, a community's familiarity with a species can also lead to ex situ conservation, which can eventually aid rehabilitating and reintroducing threatened species into the wild (Abeli *et al*., 2020; Cochrane *et al*., 2007). Similarly, a lack of observation does not mean a species is absent (Alberta Native Plant Council [ANPC], 2012). The first sighting of *A. boscawenii* was recorded in six decades along the coast of the Boma ward in the Tanga region. Nevertheless, the species is still categorized as Critically Endangered, and several coastal human activities threaten its existence according to the findings.

Most of the *Aloe* species were patchily distributed in rocky areas, i.e. *A. ballyi*, *A. bicomitum*, *A. confusa*, *A. dorotheae*, *A. fibrosa*, *A. flexilifolia*, *A. lateritia* (Lushoto district), *A. leptosiphon*, *A. mzimbana*, *A. myriacantha*, *A. nuttii*, and *A. volkensii*. Rocks are sheltering ground away from human disturbance and threats like fires (Arena *et al*., 2015; Larson *et al*., 2005). However, the inherently barren environments such as rocky outcrops and cliffs, do not generally support high plant growth and contribute to low population densities (Larson *et al*., 2005). Furthermore, a narrow habitat range increases a species' vulnerability to natural disasters and human activities as was found for various Tanzanian *Aloe* species. Thus, it is recommended that human activities should be restricted or prohibited in those rocky areas and cliffs where *Aloe* species are present.

The research found out that, most threatened *Aloe* species were present in high elevation areas that are also considered fertile by the local communities and, thus, more favorable for agricultural activities than moderate and low elevation areas (Hall *et al*., 2009; Winowiecki *et al*., 2016). This increases the risk of human activities that can threaten *Aloe* species in these areas. Lowlands, in this case defined as coastal areas and the land close to freshwater bodies, are mostly urbanized witha high concentration of economic activities such as fishing, salt making, tourism, and recreation. These activities also threaten the locally available *Aloe* species habitat. The *Aloe* species along the shorelines are further at risk due to dynamic and unpredictable weather conditions such as hurricanes and floods (Ouborg *et al*., 2006).

The highest number of threatened *Aloe* species was found within the study group in the Eastern Arc Mountains (South and North Pare, East and West Usambara), Coastal Forests, and the Katavi-Rukwa ecosystem. The EAM has been documented to be very rich in endemic species compared to other Eastern Africa areas (Hall *et al*., 2009; Howell *et al*., 2006). There are different land use categories in Tanzania and 7000 km^2 are in a protected area made up of national parks, nature reserves, and forest reserves (Burgess & Kilahama, 2005). Most of the threatened *Aloe* species were found within these protected areas. This is because, the *Aloe* populations are diminishing in less strongly protected areas due to over-exploitation, land-use change, and poaching in the EAM (Kideghesho & Msuya, 2010; Tabor *et al*., 2010), coastal forests (Godoy *et al*., 2011; Tabor *et al*., 2010), and the Katavi-Rukwa ecosystem (Wilfred *et al*., 2019). More research on *Aloe* species distribution and threats is needed in other areas of the Eastern Arc Mountains (EAM), including North and South Nguru, Uluguru, Ukaguru, Rubeho, Malundwe, Udzungwa, Mahenge, and Uvidunda Mountains.

(ii) Threat and Conservation of *Aloe* Species

There was no strong correlation between high utilization of *Aloe* species and its threat level. Moreover, the most-utilized *Aloe* species, *A. duckeri*, *A. lateritia*, and *A. secundiflora*, are non-endemic, widely distributed and categorised as Least Concern. In the present re-assessment, all three *Aloe* species were assessed as Least Concern in Tanzania, as they are considered worldwide in the IUCN Red List (Richart, 2019a; Weber, 2013; Weber & Demissew, 2013b). The high number of locations and EOO explains this, although the survey was limited to Tanzania only. In Kenya, the EOO was above 20 000 km^2 for *A. deserti*, *A. lateritia* var. *lateritia*, *A. secundiflora*, and *A. volkensii*. In contrast to the situation in Tanzania (Table 1), in Kenya these species are, therefore, not considered threatened with the exception of *Aloe lateritia* var. *graminicola* (Reynolds) S. Carter, which was categorised as Vulnerable (Wabuyele, 2006).

Although interviewees reported moderate use levels for *A. flexilifolia* and *A. leptosiphon*, they were categorised as Endangered. For both species, previous assessments (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009i, 2009m) mentioned that they could be impacted by collectors. For *A. flexilifolia*, two respondents in the study also observed the same. This is likely one reason for their current threat category. However, despite these examples to the contrary, it can be hypothesized that the current threat level for most *Aloe* species is due to their restricted distribution range and not to human use, i.e. *A. confusa* and *A fibrosa*.

There was always no agreement between the species-specific folk perception on the availability of *Aloe* species and the threat level that were assigned using number of locations, EOO and AOO. While the 77% of the studied *Aloe* species were assigned as threatened, the majority of respondents viewed 45% of the species to be available in the wild. This difference between local community perceptions and scientists is well-known (Sajem *et al*., 2008). For example, here a community perceived the Least Concern and widespread *A. duckeri*, *A. lateritia,* and *A. myriacantha* to be threatened. Even though *A. lateritia* has a large EOO (624 760 km^2), it was scarcely available compared to other species at the village level. This is because communities tend to look at a species' local status when defining its availability, while scientists more commonly use larger geographical areas.

The conservation status of the Critically Endangered *A. boscawenii* and *A. fibrosa* is in agreement with the community perception of its availability. The endangered status of *A. boscawenii* is attributed to its restricted distribution range Human activities were also observed along the coast that likely affect *A. boscawenii* population levels, including salt harvesting adjacent to one location which was also reported in the previous assessment (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b), recreational activities and trampling by fishermen as they hide their fishing gear. Seven of the *Aloe* species that were categorized as Endangered (*A. bicomitum*, *A. chabaudii*, *A. deserti*, *A. flexilifolia*, *A. parvidens*, *A. rabaiensis*, and *A. volkensii*) in this study, and two that were assessed as Vulnerable (*A. dorotheae* and *A. macrosiphon*) were perceived by the local communities as available.

Despite some disagreements, involving locals in plant species assessments is often essential due to their in-depth knowledge on how to find and use them (Biró *et al*., 2014). This was also reported in Benin, where traditional knowledge of woody plant species was essential in identifying the highly threatened ones, i.e. *Afzelia africana* Pers., *Khaya senegalensis* (Desv.) A. Juss., *Milicia excelsa* (Welw.) C. C. Berg, and *Pterocarpus erinaceus* Poir. (Agbani *et al*., 2018). Traditional knowledge is also often relevant for managing natural resources for conservation purposes (Liu *et* *al*., 2002). Thus, taking communities' perceptions on availability and threat factors into account when considering conservation policies for *Aloe* species is recommended.

A major contributing factor towards declining levels of natural vegetation worldwide is land-use changes (Maundu *et al*., 2006). This study found out that the studied *Aloe* species were affected by agricultural activities encroaching in natural habitats, urbanization such as roads and buildings, overharvesting, and land clearing. Fire for land preparation had effect on *A. dorotheae*, which usually grows in rocky areas far away from agricultural fields. However, *Aloe* species have developed different adaptation mechanisms to withstand fires, including changes in their succulent nature (Cousins & Witkowski, 2012), increased insulation resulting from attached dead leaves (Bond, 1983) and refuge on rocky areas (Larson *et al*., 2005; Pfab & Witkowski, 1999). Nevertheless, these adaptations can negatively affect pollination rate, seed set, and seedling survival, which in turn reduces next-generation population levels (Cousins & Witkowski, 2012). Additionally, livestock and wild animals have also been reported to damage *Aloe* species (Cousins & Witkowski, 2012; Newton, 2004). Policy makers can use the present study as an aid in deciding which threatened species to priorities in conservation efforts. Since the number of species threatened with extinction outweighs the resources available for conservation, making informed decisions on which species to prioritize is essential (Myers *et al*., 2000).

(iii) *Aloe* Species and Temperature

In this study, most *Aloe* species germinated at moderate temperatures of 25°C and 30°C and germination success declined at a higher temperature (35°C) supported by other researchers (Kulkarni *et al.,* 2013). The optimum temperature for germination was reported to be 30°C, followed by a lower temperature of 25°C. *Aloe* species avoid cold conditions below 20°C (Kulkarni *et al.,* 2013; Liu *et al.*, 2011) but some studies claimed that germination could also be low under high temperatures, i.e., from 30°C upwards (Bairu *et al.,* 2009; Kulkarni *et al.,* 2013). Hence, the rise in temperature might have a negative effect on the existence of many *Aloe* species that prefer moderate and low temperatures. Alternatively, a plant species can tolerate the rise in temperature if it is within its temperature range (Davis *et al.,* 2005) or can shift towards an area within its temperature range as was observed for the tree-like *Aloe dichotoma* in the Namib desert (Foden, 2002; Foden *et al.,* 2007). However, *Aloe* population declines and, consecutively, extinctions due to higher temperatures have also been observed (Foden *et al.,* 2007). *Aloe pembana,* a species that tolerated both medium and high temperatures usually grew on rocky areas along the coast in this study. This exposes the species to heat during the day. On the other hand, *A. secundiflora* seeds have thrived both at high and low temperatures because this species tends to occur in both warm (Dodoma) and cold (Kilimanjaro) regions of Tanzania (Carter, 1994).

(iv) *Aloe* Species and Light

Furthermore, it was observed that darkness stimulates germination (except for *A. boscawenii*) as opposed to light conditions. This might be due to the shade that parent plants and the dead leaves cast on their fallen seeds as was seen for other plant species growing in tropical environments (Cousins & Witkowski, 2012; Kulkarni *et al.,* 2006). In contrast, *A. boscawenii* has slender leaves facing up or hanging down on sea cliffs (direct observation), so growing in the shade did not result in an evolutionary advantage, and this was also seen in *A. arborescens* (Kulkarni *et al.,* 2014). The results are supported by a study on *A. ferox* (Bairu *et al.,* 2009) and *A. greatheadii* var. *davyana* (Smith & Correia, 1992) that germinated well under dark conditions. Hence, diverse *Aloe* species will react differently when exposed to various light conditions.

(v) *Aloe* Species and Scarification

Additionally, *Aloe* seeds germinated well when un-scarified. The little positive effect of scarification on germination might indicate that most *Aloe* seeds are dispersed by wind and not by seed predators (Cousins *et al.,* 2014; Symes, 2012; Wilson *et al.,* 2009). Although animals and insects have been reported to be crucial in seed germination through scarification process, they have been reported to damage the *Aloe* species in this study and by others (Bhaludra *et al.,* 2013; Breebaart *et al.,* 2002; Cousins *et al.,* 2013; Wiseman, 2001). In contrast, wind-dispersed seeds will generally result in a higher survival rate of the seeds and *Aloe* species populations (Cousins *et al.,* 2013). However, for the unwinged seeds, i.e., *A. pembana*, the dispersal and establishment of seedlings is likely close to the mature plants (Letsara *et al.,* 2012; Stokes & Yeaton, 1995).

(vi) Growth Medium for *Aloe* Species

In this study, low KNO_3 concentrations (0.01 mg/L) favoured the germination of *Aloe* species as supported by other researchers (Kulkarni *et al.,* 2013), indicating that *Aloe* seeds have adapted well to conditions of low soil fertility. Potassium nitrate has also been described to improve the concentration of flavonoids and phenolics compounds in *Aloe* species (Kulkarni *et al.,* 2013) and to counteract the hostile effect of sodium chloride for the salt-tolerant plants (Akram *et al.,* 2007; Hasanuzzaman *et al.,* 2018; Kaya *et al.,* 2007). Therefore, the tolerance of *Aloe* species that are found along the coast such as *A. boscawenii* and *A. pembana* towards a high concentration of potassium nitrate (0.1 mg/L) can be explained by an adaptive mechanism for osmosis regulation in the cell membranes, which was also documented for other *Aloe* species (Cardarelli *et al.*, 2013).

The findings that germination percentage was increased after the seeds were recovered from saline water highlights that, germination of coastal *Aloe* species occurs most likely after the rains. This happens when the salt is washed from the seeds, resulting in the seed viability recovery. As most of the coastal plants are associated with poor nutrients, wind and salt spray (Calvão *et al.*, 2013), the saline conditions decrease the ability of *Aloe* to absorb important mineral elements (Cristiano *et al.*, 2016). The negative effect of salt on germination for tested *Aloe* species has also been reported for *A. barbadensis* and *A. arborescens* (Cardarelli *et al.*, 2013). The three *Aloe* species (*A. lateritia*, *A. pembana* and *A. boscawenii*) that germinated after being recovered from saline water indicate their salt tolerance. The two coastal *Aloe* species (*A. pembana* and *A. boscawenii*) are expected to germinate better under salt conditions, but it was not expected that *A. lateritia* which is an inland species would germinate better under salt conditions. *Aloe lateritia* germination was twice as high as that for *A. pembana* and seven times higher than *A. boscawenii* under salinity conditions. This suggests high habitat tolerance of the LC species such as *A. lateritia.*

Furthermore, the germination percentage and sprouts' initiation were slightly higher on filter paper water medium on the soil as supported by other studies (Berthold, 2014). For cultivation of *Aloe* species in the future, germination of *Aloe* seeds is suggested to be done on filter paper with distilled water medium and later a transfer of the seedlings into the soil medium to increase the number and pace of seeds germinating.

The CR species, *A. pembana*, exhibited a higher germination percentage in all treatments compared to *A. boscawenii*. The higher germination of *A. pembana* seeds suggests the species' greater adaptability to various environmental conditions such as its ability to withstand different temperatures and KNO_3. In contrast, the low performance/germination of *A. boscawenii* across all treatments compared to *A. pembana* might be due to dormancy, as had also been reported for *A. verecunda* Pole-Evans (Craib, 2007). *Aloe pembana* has been documented and observed in this study to produce side shoots on the upper section of its stem, thereby promoting vegetative growth (Plate 4; Cousins & Witkowski, 2012). This explains the better performance and persistence of *A. pembana* in the long run compared to *A. boscawenii*. Vegetative reproduction has been regarded as an outstanding approach in increasing the survival potential of a species (Alam & Ali, 2010). Nevertheless, *A. pembana* is still CR as it is associated with a limited, restricted range (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009h). The *Aloe* species tested responded according to the predictions based on their IUCN Red List status (except for *A. pembana*), highlighting the importance of their protection in the future.

(vii) *Aloe* Species-Specific Responses

The *Aloe* species tested in this study showed species-specific responses to different treatments, which reflected their current Red List status, except for *A. pembana*. Generally, *A. lateritia*, a species of LC (IUCN Red List) outperformed all other species in all experimental treatments, except at temperature conditions of 35°C and 30°C. This species was followed by the CR *A. pembana* and the LC *A. secundiflora*. The results suggest that the rareness of *A. pembana* might not be related to germination as it was postulated before because it germinated better than the LC *Aloe* species. This peculiar seed form suggests that the seeds are not wind-dispersed, which could explain the species' extremely limited distribution (Campbell-Barker, 2012) with dense seedling stands in close proximity to the parent plant. The results call for further studies on *A. pembana* distribution via herbivorous species on Pemba island, Tanzania.

The species of LC, *A. lateritia*, outperformed other species across all treatments in its germination success, followed by *A. secundiflora* and *A. volkensii*. This is supported by other researchers who reported the first two species being widely distributed across different ographical areas of various climatic conditions (Bjorå *et al.*, 2015; Carter, 1994; Wabuyele, 2006). *Aloe volkensii* has a greater risk of becoming classified as a threatened species due to its narrow tolerance based on the tests on environmental parameters affecting germination.

Plate 4: *Aloe pembana* young vegetative shoot on the side of its upper stem at Pemba island, December 2018, Photo's by Siri Abihudi

4.2.6 In-vitro culture regeneration of *A. boscawenii*

In this study, the ideal concentration for surface sterilization for *A. boscawenii* was NaOCl (6%) while Hg $(NO_3)_2$ reduced contamination but no proliferation, although the different concentration of NaOCl (Abrie & van Staden, 2001; Chukwujekwu *et al.,* 2002; Hashemabadi & Kaviani, 2008; Molsaghi *et al.*, 2014; Zakia *et al.,* 2013) and 0.1% $HgCl_2$ (Ahmed *et al.*, 2007; Baksha *et al.*, 2005; Zakia *et al.*, 2013) have been used in surface sterilization of *Aloe* species worldwide. The high concentration of NaOCl and small for $Hg(NO_3)_2$ result in drying of the samples. The percentage number of uncontaminated explants tend to reduce when concentration increase but end up not proliferating. The use of Hg $(NO_3)_2$ resulted from the unavailability of $HgCl_2$. The application of Hg is currently discouraged due to its hazardous effect on human and the environment (Kaviani & Branch, 2015). The high level of contamination during culture initiation attributed to explant material growing in nature (Bunn, 2002).

Additionally, the primary source of contamination was from endophytes fungus as it revealed after the use of an antifungal agent. These endophytic fungal did not hinder the growth of the cultured materials after proliferation. Such mutualistic relationships are common in different plant species where the microbes form antagonistic activities against the enemy of their host. Examples, the endophytes tend to fight the microbes causing diseases and promotes plants resistant biotic and abiotic stresses (Abdalla & Matasyoh, 2014; Rai *et al.*, 2014; Rajamanikyam *et al.,* 2017). In some plant, such a banana cultivars Enyeru and Kibuzi, tissue culture application resulted with sterile materials that lost all the good endophytes against weevils and parasitic nematodes (Dubois *et al.*, 2004). The cultured materials of *A. boscawenii* were able to grow in the endophytic fungus present in which they later fed inside the plant during the multiplication stage. Such a relationship can influence the production of secondary metabolites of the host plant (Abdalla & Matasyoh, 2014; Jalgaonwala *et al.,* 2011; Rajamanikyam *et al.,* 2017) are the potential source of medicine to human beings. On the other hand, the contamination from bacteria might result from the medium and not the plant itself.

In my research work, seeds performed better during the proliferation stage than other explant materials, although it failed to multiply as it was for *Aloe pretoriensis* Pole-Evans (Groenewald *et al.,* 1975). The survival frequency of cultured *Aloe* seeds seems to be low even for other species, i.e. *Aloe polyphylla* due to browning and hyperhydricity (Abrie & van Staden, 2001). Moreover, the seed germination percentage reached for tissue culture was almost similar to traditional germination experiment. The CR Red List status for *A. boscawenii* (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants, 2009b) is linked to low seed viability which hinders its sexual propagation and spread. On the other hand, meristems, shoot tip and taproots are the ideal explant material for *A. boscawenii* tissue culture proliferation and multiplication as it was for *A. barbadensis* meristem (Sanchez *et al.*, 1988; Natali *et al.*, 1990) and *A. vera* shoot tip (Ahmed *et al.*, 2007). It was surprising for the taproot to proliferate and multiply as it has not mentioned in literature for other *Aloe* species. Hence, the explant material choice is crucial as it has a very high contribution to the proliferation and multiplication of explant materials during the initiation stage (Abadi & Kaviani, 2010).

The tissue injury can ascribe browning during proliferation and multiplication stage due to the metabolism of a phenolic leak that might follow from cutting (Baskaran *et al.*, 2015; Bunn, 2002). Browning was not a problem in my study as it did not affect the growth and multiplication stage of the culture materials which was not the case by Baskaran *et al.* (2015) were it affected germination and conversion of culture material from somatic embryos. In the management of browning, the PVP (Abrie & van Staden, 2001; Gantait *et al.*, 2014; Hashem & Kaviani, 2010; Molsaghi *et al.*, 2014), ascorbic acid (Abrie & van Staden, 2001; Amoo *et al.*, 2012; Roy & Sarkar, 1991), citric acid (Hashem & Kaviani, 2010; Molsaghi *et al.*, 2014; Nayanakantha *et al.*, 2011) or activated charcoal (Kiran *et al.*, 2017; Nayanakantha *et al.*, 2011) can be applied successfully.

Rooting with NAA gave intertwined root system, which gave them tremendous strength when acclimatized with the outside environment while IBA gave slender and long roots. The combination NAA with half of IBA is the ideal rooting media for *A. boscawenii* as it provides both long and robust root system. Moreover, the respective rooting hormone and their combination did not significantly affect the number of shoots produced during the rooting phase as it was for *A. arborescens* (Amoo *et al.*, 2012; Molsaghi *et al.*, 2014) where the combination performed best. Acclimatization of *A. boscawenii* was highly successful in the mixture (Sand + Sawdust + Coco peat media) and on sand compared to other media. This combination did not give a very light medium as suggested by abadi and Kaviani (2008), although it had a good drainage atmosphere. Moreover, hardening plantlets with a strong root system was done on a mixture of sand and cow manure. Other studies have suggested the use of sand and soil (1:1) or the combination of sand, soil and perlite (1:1:1) for hardening of *A. vera* (Natali *et al.*, 1990).

Furthermore, the mature plantlets produce through tissue culture had no morphological abnormalities. It is assumed that the acclimatization and hardening stage's success can only depend on the un-interfered morphological structure of the plantlets. It can be concluded that the application of tissue culture is supreme techniques in the restoration of CR species as it gave the highest number of plantlets compared with traditional propagation (seed germination and cuttings).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study shows that species in the genus *Aloe* have numerous health and therapeutic uses across Tanzania. Although, most *Aloe* species in Tanzania are declining based on both quantitative and qualitative informants' perceptions survey. There is a ubiquitous threat to *Aloe* species in high and low elevations, from the Eastern Arc Mountains to Coastal Forests. *Aloe* species that cluster together and find refuge in rocky areas are frequently assessed as threatened due to their greater vulnerability towards human activities including agriculture, fire, overharvesting and climate change. That most of the *Aloe* species surveyed are threatened even in protected areas such as the Eastern Arc Mountains and Katavi-Rukwa ecosystems, the fate of the species is unwarranted. In *Aloe* species' conservation, the seeds germinate best under moderate temperature (25^{o}C and 30^{o}C) and low potassium nitrate (0.01 mg/L). The increase in temperature and potassium nitrate had a negative effect on most *Aloe* species seed germination. Saline water resulted in seed dormancy of all *Aloe* seeds tested, with viability being recovered when the *Aloe* seeds were washed with distilled water medium. The dark conditions stimulate germination, highlighting the importance of leaf litter, parent plants or a healthy canopy cover in the germination habitat. Understanding the germination behaviour in response to various environmental conditions is essential in ex-situ conservation of *Aloe* species as the suitable growing conditions can be provided. Furthermore, in-situ conservation of *A. boscawenii* can be done through cutting in small scale and through tissue culture in large scale.

5.2 Recommendations

The ethnobotanical uses of *Aloe* species indicate the need for pharmacological studies to determine the type of chemical compounds found in *Aloe* extracts for potential clinical and commercial application. Although *Aloe* plants and plant parts were of low commercial value in the study area, local authorities should control their harvest by traditional healers and registered and unregistered companies. Failing to take these conservation efforts could have detrimental effects on the abundance of several species in the genus. Hence, there is a need to update *Aloe* species' conservation assessments continually as their available habitat continuously changes.

A more concerted effort to locate remaining populations and update the *A. confusa* assessment is needed since no plants was found along Kifaru river. The study calls for more in situ conservation efforts of riparian ecosystems to protect *Aloe* and other species. This requires more application of natural resources laws and policies, including *Aloe* species, because the decline in *Aloe* species is higher than previously thought.

However, to ensure *Aloe* species' future availability, given their high medicinal value, conservation needs must be addressed. Information provided in this study on the germination of *Aloe* species can be used for the genus' ex-situ conservation. Since the medicinal *Aloe* species' cultivation was not expected, there is a need to train local people on domestication techniques. This can be done through community farming of useful medicinal *Aloe* species by local people, thus reducing pressure onto the wild populations. This study is as well crucial for *Aloe* species population development trajectories. It can provide necessary information for future species distribution models under a raising global temperature and more rainfall extremes in climate change. For the *Aloe* species such as *A. boscawenii,* that had little FGP across all treatment, other techniques such as propagation through cuttings and in vitro culture can be applied. The germination trials will be best used by local communities for conservation of *Aloe* species on a small scale, such as in a home garden. Tissue culture can be used on a large scale, such as in industrial and commercial production.

REFERENCES

Abadi, H. D., & Kaviani, B. (2010). In vitro proliferation of an important medicinal plant Aloe- A method for rapid production. *Australian Journal of Crop Science*, *4*(4), 216–222. https://www.researchgate.net/publication/285796349

Abadi, H. D., & Kaviani, B. (2008). Rapid micro-propagation of *Aloe vera* L. via shoot multiplication. *African Journal of Biotechnology*, *7*(12), 1899–1902. https:// doi. org/ 10. 5897/ AJB2008.000-5038

Abdalla, M. A., & Matasyoh, J. C. (2014). Endophytes as Producers of Peptides: An Overview About the Recently Discovered Peptides from Endophytic Microbes. *Natural Products and Bioprospecting*, *4*(5), 257–270. https://doi.org/10.1007/s13659-014-0038-y

Abeli, T., Dalrymple, S., Godefroid, S., Mondoni, A., Müller, J. V., Rossi, G., & Orsenigo, S. (2020). Ex situ collections and their potential for the restoration of extinct plants. *Conservation Biology*, *34*(2), 303–313. https://doi.org/10.1111/cobi.13391

Abeli, T., Gentili, R., Rossi, G., Bedini, G., & Foggi, B. (2009). Can the IUCN criteria be effectively applied to peripheral isolated plant populations? *Biodiversity and Conservation*, *18*(14), 3877–3890. https://doi.org/10.1007/s10531-009-9685-4

Abrie, A. L., & Van Staden, J. (2001). Micropropagation of the endangered *Aloe polyphylla*. *Plant Growth Regulation*, *33*(1), 19–23. https://doi.org/10.1023/A:1010725901900

Adelberg, J., & Naylor-adelberg, J. (2012). Effects of Cytokinin on Multiplication and Rooting of *Aloe barbadensis* during Micropropagation on Agar and Liquid Medium. *Journal of Medicinally Active Plants*, *1*(1), 1–5. https://doi.org/10.7275/R5251G4V

Agbani, P. O., Kafoutchoni, K. M., Salako, K. V., Gbedomon, R. C., Kégbé, A. M., Karen, H., & Sinsin, B. (2018). Traditional ecological knowledge-based assessment of threatened woody species and their potential substitutes in the Atakora mountain chain, a threatened hotspot of biodiversity in Northwestern Benin, West Africa. *Journal of Ethnobiology and Ethnomedicine*, *14*(1), 1–19. https://doi.org/10.1186/s13002-018-0219-6

Aggarwal, D., & Barna, K. S. (2014). Tissue culture propagation of elite plants of Tissue Culture Propagation of Elite Plant of *Aloe vera* Linn. *Journal of Plant Biochemistry and Biotechnology*, *13*, 77–79. https://doi.org/10.1007/BF03263197

Ahmed, S., Kabir, A., Ahmed, M., Razvy, M., & Ganesan, S. (2007). Development of rapid micropropagation method of *Aloe vera* L. *Seed Science Journal*, *24*(2), 121–128. https://www.researchgate.net/publication/209843942

Akeroyd, J. (2002). A rational look at extinction. *Plant Talk*, *28*, 35–37.

Akram, M. S., Athar, H. U. R., & Ashraf, M. (2007). Improving growth and yield of sunflower (*Helianthus annuus* L.) by foliar application of potassium hydroxide (KOH) under salt stress. *Pakistan Journal of Botany*, *39*(3), 769–776. https:// www. researchgate. net/ publication/ 267938870

Alam, J., & Ali, S. I. (2010). Conservation status of *Androsace russellii* Y. Nasir: A critically endangered species in Gilgit district, Pakistan. *Pakistan Journal of Botany*, *42*(3), 1381–1393. https://www.researchgate.net/publication/33293400

Alberta Native Plant Council. (2012). *ANPC Guidelines for Rare Vascular Plant Surveys in Alberta - 2012 Update*. *April*, 25. http://anpc.ab.ca/wp-content/ uploads/ 2015/ 01/ Guidelines- For-Rare-Plant-Surveys-in-AB-2012-Update.pdf

Amir, H. M., Grace, O. M., Wabuyele, E., & Manoko, M. L. K. (2019). Ethnobotany of *Aloe* L. (*Asphodelaceae*) in Tanzania. *South African Journal of Botany*, 122, 330-335. https:// doi. org/10. 1016/j. sajb. 2019.01.038

Amoo, S. O., Aremu, A. O., & Van Staden, J. (2012). In vitro plant regeneration, secondary metabolite production and antioxidant activity of micropropagated *Aloe arborescens* Mill. *Plant Cell, Tissue and Organ Culture*, *111*(3), 345–358. https://doi.org/10.1007/s11240-012-0200-3

Angiosperm Phylogeny Group. (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, *161*, 105–121. https://doi.org/10.1111/boj.12385

Araujo, D. S. D., & Pereira, M. C. (2007). Sandy coastal vegetation. *Encyclopedia of Life Support Systems (EOLSS); International Commission on Tropical Biology and Natural Resources. EOLSS Publishers Co. Ltd., Oxford, UK*, *IV*. https:// www. researchgate. net/ publication/ 267422451

Arena, G., Symes, C. T., & Witkowski, E. T. F. (2013). The birds and the seeds: Opportunistic avian nectarivores enhance reproduction in an endemic montane aloe. *Plant Ecology*, *214*(1), 35–47. https://doi.org/10.1007/s11258-012-0144-z

Arena, G., Witkowski, E. T. F., & Symes, C. T. (2015). Growing on rocky ground: Microhabitat predictors for site-occupancy of *Aloe peglerae*, an Endangered endemic species with a restricted range. *South African Journal of Botany*, *100*, 174–182. https:// doi. org/ 10.1016/ j.sajb.2015.05.022

Awang, N. A., Ali, A. M., & Mat, N. (2018). Alternative medicines from edible bitter plants of Besut, Malaysia. *Journal of Agrobiotechnology*, *9*(2), 80–91. https:// journal. unisza. edu. my/agrobiotechnology/index.php/agrobiotechnology/article/view/134

Bachman, S., Moat, J., Hill, A. W., De laTorre, J., & Scott, B. (2011). Supporting red list threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys*, 150, 117–126. https://doi.org/10.3897/zookeys.150.2109

Bachman, S. P., Wilkin, P., Reader, T., Field, R., Weber, O., Nordal, I., & Demissew, S. (2020). Extinction risk and conservation gaps for *Aloe* (*Asphodelaceae*) in the Horn of Africa. *Biodiversity and Conservation*, *29*(1), 77–98. https://doi.org/10.1007/s10531-019-01870-0

Bairu, M. W., Kulkarni, M. G., Street, R. A., Mulaudzi, R. B., & Van Staden, J. (2009). Studies on seed germination, seedling growth, and in vitro shoot induction of *Aloe ferox* mill., a commercially important species. *HortScience*, *44*(3), 751–756. https:// doi. org/10. 21273/ hortsci. 44.3.751

Baksha, R., Ara, M., Jahan, A., Khatun, R., & Munshi, J. L. (2005). Micropropagation of *Aloe barbadensis* Mill. through In vitro Culture of Shoot tip Explants. *Plant Tissue Culture and Biotechnology*, *15*(2), 121–126. https://www.researchgate.net/publication/288943028

Bani, P., Grossi, P., Lucini, L., Pellizzoni, M., Minuti, A., & Trevisi, E. (2016). Administration of *Aloe arborescens* homogenate to cattle: Interaction with rumen fermentation and gut absorption of aloin. *Italian Journal of Animal Science, 15*(2), 233–240. https:// doi. org/ 10.1080/ 1828051X.2016.1157007

Barnea, A., Yom-Tov, Y., & Friedman, J. (2006). Does Ingestion by Birds Affect Seed Germination? *Functional Ecology*, *5*(3), 394–402. https://doi.org/10.2307/2389811

Baskaran, P., Kumari, A., Naidoo, D., & Staden, J. (2015). In vitro propagation and biochemical changes in *Aloe pruinosa*. *Industrial Crops and Products*, *77*, 51–58. https://doi.org/10.1016/j.indcrop.2015.08.036

Basmatker, G., Jais, N., & Daud, F. (2011). *Aloe vera*: A valuable multifunctional cosmetic ingredient. *International Journal of Medicinal and Aromatic Plants*, *1*(3), 338–341. http://www.openaccessscience.com

Baumann, B. B., & William, S. (1960). The Botanical Aspects of Ancient Egyptian Embalming and Burial. *Economic Botany*, *16*(1), 84–85. https://www.jstor.org/stable/4252139

Berlin, B. (1973). Folk Systematics in Relation to Biological Classification and Nomenclature. *Annual Review of Ecology and Systematics*, *4*(1), 259–271. https:// doi. org/ 10. 1146/ annurev.es. 04.110173.001355

Berlin, B., Breedlove, D. E., & Raven, P. H. (1966). Folk taxonomies and biological classification. *Science*, *154*(3746), 273–275. https://doi.org/10.1126/science.154.3746.273

Berthold, C. (2014). An investigation into improving the germination rate of *Aloe polyphylla* schonland ex pillans. *The Journal of Botanic Garden Horticulture*, *12*(12), 139–154. https://hdl.handle.net/20.500.12594/1784

Bhaludra, C. S. S., Bethapudi, R. R., Murugulla, A. C., Pullagummi, C., Latha, T., Venkatesh, K., Bheemagani, A. J., Pudutha, A., & Rani, A. R. (2013). Cultivation, phytochemical studies, biological activities and medicinal uses of *Aloe ferox*, grandfather of Aloes an important amazing medicinal plant. *International Journal of Pharmacology*, *9*(7), 405–415. https://doi.org/10.3923/ijp.2013.405.415

Biró, É., Babai, D., Bódis, J., & Molnár, Z. (2014). Lack of knowledge or loss of knowledge? Traditional ecological knowledge of population dynamics of threatened plant species in East-Central Europe. *Journal for Nature Conservation*, *22*(4), 318–325. https:// doi. org/ 10. 1016/ j.jnc.2014.02.006

Bjorå, C. S., Wabuyele, E., Grace, O. M., Nordal, I., & Newton, L. E. (2015). *The uses of Kenyan aloes: An analysis of implications for names, distribution and conservation*. https://www.google.com

Bland, L. M., Keith, D. A., Miller, R. M., Murray, N. J., & Rodríguez, J. P. (2016). *Guidelines for the application of IUCN Red List of Ecosystems Categories and Criteria* (Version 1.). Gland, Switzerland: IUCN. ix + 94pp. http://dx.doi.org/10.2305/IUCN.CH.2016.RLE.1.en

Bogler, D. J., Pires, J. C., & Francisco-Ortega, J. (2005). Phylogeny of *Agavaceae* based on ndhf, rbcl, and its sequences: Implications of molecular data for classification. *Aliso The Rancho Santa Ana Botanic Garden*, *22*(1), 311–326. https://doi.org/10.5642/aliso.20062201.26

Bonar, S., Fehmi, J., & Mercado-Silva, N. (2010). *An overview of sampling issues in species diversity and abundance surveys*. https://www.google.com

Bond, W. (1983). Dead leaves and fire survival. *Oecologia*, *58*(1), 110–114. https://doi.org/10.1007/bf00384549

Botes, C., Johnson, S. D., & Cowling, R. M. (2008). Coexistence of succulent tree aloes: Partitioning of bird pollinators by floral traits and flowering phenology. *Oikos*, *117*(6), 875–882. https://doi.org/10.1111/j.0030-1299.2008.16391.x

Botes, C., Johnson, S. D., & Cowling, R. M. (2009). The Birds and the Bees: Using Selective Exclusion to Identify Effective Pollinators of African Tree Aloes. *International Journal of Plant Sciences*, *170*(2), 151–156. https://doi.org/10.1086/595291

Breebaart, L., Bhikraj, R., & O'connor, T. G. (2002). Impact of goat browsing on *Aloe ferox* in a South African savanna. *African Journal of Range and Forage Science*, *19*(1), 77–78. https://doi.org/10.2989/10220110209485777

Brown, J. H. (1984). On the relationship beween abundance and distribution of species. *American Naturalist*, *124*(2), 255–279. https://doi.org/10.1086/284267

Brummitt, N., Bachman, S. P., & Moat, J. (2008). Applications of the IUCN Red List: Towards a global barometer for plant diversity. *Endangered Species Research*, *6*(2), 127–135. https://doi.org/10.3354/esr00135

Bunn, E. (2002). *Microbial contaminants in plant tissue culture propagation*. https:// www. google. com

Burgess, N. D., & Kilahama, F. (2005). Is enough being invested in Tanzania's Eastern Arc Mountains? *The Arc Journal*, *17*, 2–5. https://www.researchgate.net/publication/312985045

Cabahug, R. A. M., Nam, S. Y., Lim, K. B., Jeon, J. K., & Hwang, Y. J. (2018). Propagation Techniques for Ornamental Succulents. *Flower Research Journal*, *26*(3), 90–101.

https://doi.org/10.11623/frj.2018.26.3.02

Calvão, T., Pessoa, M. F., & Lidon, F. C. (2013). Impact of human activities on coastal vegetation: A review. *Emirates Journal of Food and Agriculture*, *25*(12), 926–944. https://doi.org/10.9755/ejfa.v25i12.16730

Campbell-Barker, T. (2012). Notes on *Aloe canis* - a new species found in Malawi. *The Society of Malawi Journal*, *65*(1), 20–27. https://www.jstor.org/stable/23342082

Cardarelli, M., Rouphael, Y., Rea, E., Lucini, L., Pellizzoni, M., & Colla, G. (2013). Effects of fertilization, arbuscular mycorrhiza, and salinity on growth, yield, and bioactive compounds of two *Aloe* species. *HortScience*, *48*(5), 568–575. https:// www. researchgate. net/ publication/262261678

Carter, S., Lavranos, J. J., Newton, L. E., & Walker, C. C. (2011). *Aloe: The Definitive Guide*. Royal Botanical Garden. https://www.google.com

Carter, S. (1994). *Aloaceae: Flora of Tropical East Africa*. Royal Botanical Gardens, Kew. https://www.google.com

Castle, L. M., Leopold, S., Craft, R., & Kindscher, K. (2014). Ranking tool created for medicinal plants at risk of being overharvested in the wild. *Ethnobiology Letters*, *5*(1), 77–88. https://doi.org/10.14237/ebl.5.2014.169

Castorena S., I., Natali, L., & Cavallin, A. (1988). In vitro culture of *Aloe barbadensis* Mill.: Morphogenetic ability and nuclear DNA content. *Plant Science*, *55*(1), 53–59. https://doi.org/10.1016/0168-9452(88)90041-6

Çavusoğlu, D., Tabur, S., & Çavusoğlu, K. (2016). The effects of *Aloe vera* L. Leaf extract on some physiological and cytogenetical parameters in *Allium cepa L.* Seeds germinated under salt stress. *Cytologia*, *81*(1), 103–110. https://doi.org/10.1508/cytologia.81.103

Chase, M. W., Christenhusz, M. J. M., Fay, M. F., Byng, J. W., Judd, W. S., Soltis, D. E., Mabberley, D. J., Sennikov, A. N., Soltis, P. S., Stevens, P. F., Briggs, B., Brockington, S., Chautems, A., Clark, J. C., Conran, J., Haston, E., Möller, M., Moore, M., Olmstead, R., … Weber, A. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, *181*(1), 1–20. https://doi.org/10.1111/boj.12385

Chukwujekwu, J. C., Fennell, C. W., & Van Staden, J. (2002). Optimisation of the tissue culture protocol for the endangered *Aloe polyphylla*. *South African Journal of Botany*, *68*(4), 424–429. https://doi.org/10.1016/S0254-6299(15)30368-9

CITES. (2003). Review of significant trade in East African Aloes. In *CITES. PC14 Do.9.2.2. Annex*. http:// scholar. google. com/ scholar? q=Review+ of+ significant+ trade+ in+ East+ African+ Aloes&btnG=&hl=en&as_sdt=0,5#0

CITES. (2019). Appendices I, II, and III. *Convention on International Trade in Endangered Species of Fauna and Flora*. http:// www. ncbi. nlm. nih. gov/ pubmed/ 19547689

Clemens, J., Jones, P. G., & Gilbert, N. H. (1977). Effect of seed treatments on germination in *Acacia* spp. *Australian Journal of Botany*, *25*(3), 269–276. https:// doi. org/ 10. 1071/ BT9770269

Cochrane, J. A., Crawford, A. D., & Monks, L. T. (2007). The significance of ex situ seed conservation to reintroduction of threatened plants. *Australian Journal of Botany*, *55*(3), 356–361. https://doi.org/10.1071/BT06173

Cocroft, R. (2005). The Behavioral Ecology of Insect Vibrational Communication. *BioScience*, *55*, 323–334. https://doi.org/10.1641/0006-3568(2005)055

Colombo, M. L., Marangon, K., Locatelli, C., & Giacchè, M. (2009). Hemlock poisoning due to plant misidentification. *Journal of Pharmaceutical Sciences and Research*, *1*(4), 43–47. https://www.researchgate.net/publication/41394907

Colson, C. R. D., & De Broe, M. E. (2005). Kidney injury from alternative medicines. *Advances in Chronic Kidney Disease*, *12*(3), 261–275. https://doi.org/10.1016/j.ackd.2005.03.006

Copeland, L. O., & McDonald, M. B. (1999). *Principles of Seed Science and Technology*. Springer Science and Bussiness Media. New York, US. https://doi.org/10.1007/978-1-4615-1783-2

Cousins, S. R., & Witkowski, E. T. F. (2012). African aloe ecology: A review. *Journal of Arid Environments*, *85*, 1–17. https://doi.org/10.1016/j.jaridenv.2012.03.022

Cousins, S. R., Witkowski, E. T. F., & Mycock, D. J. (2014). Seed storage and germination in *Kumara plicatilis*, a tree aloe endemic to mountain fynbos in the Boland, south-western Cape, South Africa. *South African Journal of Botany*, *94*, 190–194. https:// doi. org/ 10. 1016/j. sajb.2014.07.003

Cousins, S. R., Witkowski, E. T. F., Pfab, M. F., Riddles, R. E., & Mycock, D. J. (2013). Reproductive ecology of *Aloe plicatilis*, a fynbos tree aloe endemic to the Cape Winelands, South Africa. *South African Journal of Botany*, *87*, 52–65. https:// doi. org/ 10. 1016/j. sajb. 2013.02.174

Craib, C. (2007). *Aloe verecunda*. *BioOne*, *79*(1), 30–31. https://doi.org/10.2985/0007-9367(2007)79[30:AV]2.0.CO;2

Cristiano, G., Murillo-Amador, B., & De Lucia, B. (2016). Propagation Techniques and Agronomic Requirements for the Cultivation of Barbados Aloe (*Aloe vera* (L.) Burm. F.): A Review. *Frontiers in Plant Science*, *7*, 1–14. https:// doi. org/ 10. 3389/ fpls. 2016.01410

Crosswhite, F. S., & Crosswhite, C. D. (1984). *Aloe vera*, plant symbolism and the threshing floor: Light, life and good in our heritage. *Desert Plants*, *6*(1), 43–50. http:// hdl. handle. net/ 10150/552247

Cunningham, A. B. (1993). *African medicinal plants: Setting priorities in the interface of Conservation and primary healthcare*. UNESCO press. https://www.google.com

Curvetto, N., Marinangeli, P., & Mockel, G. (2006). Hydrogen peroxide in micropropagation of *Lilium*. A comparison with a traditional methodology. *Biocell*, *30*(3), 497–500. https:// www. researchgate. net/publication/6432891

Dagne, E., Bisrat, D., Viljoen, A., & Van Wyk, B. E. (2005). Chemistry of *Aloe* Species. *Current Organic Chemistry*, *4*(10), 1055–1078. https://doi.org/10.2174/1385272003375932

Davis, M. B., Shaw, R. G., & Etterson, J. R. (2005). Evolutionary responses to changing climate. *Ecology*, *86*(7), 1704–1714. https://doi.org/10.1890/03-0788

Dessalegn, F. (2006). *Taxonomic and demographic studies of on three species complexes within the Genus Aloe L. (Aloeceae) in Ethiopia*. https://www.google.com

Djoghalf, A. (2007). Secretariat of the Convention on Biological Diversity. *Biodiversity*, *8*(4), 2–2. https://doi.org/10.1080/14888386.2007.9712830

Dold, A. P., & Cocks, M. L. (2002). The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African Journal of Science*, *98*(11–12), 589–597. https:// hdl. handle. net/ 10520/EJC97419

Dorman, M., Melnikov, P., Sapir, Y., & Volis, S. (2009). Factors affecting dormancy of *Oncocyclus iris* seeds. *Israel Journal of Plant Sciences*, *57*(4), 329–333. https:// doi. org/ 10. 1560/ IJPS. 57. 4.329

Dubey, N. (2011). *Natural Products in Plant Pest Management*. CAB International, Wallingford, UK. https://www.researchgate.net/publication/298637925

Dubois, T., Gold, C. S., Coyne, D., Paparu, P., Mukwaba, E., Athman, S., & Adipala, S. K. E. (2004). Merging biotechnology with biological control: Banana Musa tissue culture plants enhanced by endophytic fungi. *Uganda Journal of Agricultural Sciences*, *9*(1), 445–451. https://hdl.handle.net/10568/103293

Eastern Arc Mountains and Coastal Forests CEPF Plant Assessment Project Participants. (2009a). *Aloe ballyi*. The IUCN Red List of Threatened Species 2009: e.T30901A9584477. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T30901A9584477.en (Vol. 8235).

Eastern Arc Mountains and Coastal Forests CEPF Plant Assessment Project Participants. (2009b). *Aloe boscawenii*. The IUCN Red List of Threatened Species 2009: e. T158325A5195623. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2009- 2. RLTS. T158325A5195623. en.

Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants. (2009c). *Aloe deserti*. The IUCN Red List of Threatened Species 2009: e. T158128A5185542. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2009-2. RLTS. T158128A5185542. en.

Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants. (2009d). *Aloe dorotheae*. The IUCN Red List of Threatened Species 2009: e. T158335A5195945. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2009-2. RLTS. T158335A5195945. en.

Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants. (2009e). *Aloe flexilifolia*. The IUCN Red List of Threatened Species 2009: e. T158247A5191712. http:// dx. doi. org/ 10. 2305/ IUCN. UK.2009-2. RLTS. T158247A5191712. en.

Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants. (2009f). *Aloe leptosiphon*. The IUCN Red List of Threatened Species 2009: e. T158284A5192905. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2009-2. RLTS. T158284A5192905. en.

Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants. (2009g). *Aloe massawana*. The IUCN Red List of Threatened Species 2009: e. T157970A5178505.

http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2009-2. RLTS. T157970A5178505. en.

Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants. (2009h). *Aloe pembana*. The IUCN Red List of Threatened Species 2009: e. T158037A5183949. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2009-2. RLTS. T158037A5183949. en.

Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants. (2009i). *Aloe rabaiensis*. The IUCN Red List of Threatened Species 2009: e.T158271A5192171. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2009-2. RLTS. T158271A5192171. en.

Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants. (2009j). *Aloe volkensii*. The IUCN Red List of Threatened Species 2009: e. T158213A5190314. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2009-2. RLTS. T158213A5190314.en.

Engelmann, F. (2004). Plant cryopreservation: Progress and prospects. *In Vitro Cellular and Developmental Biology - Plant*, 40(5), 427–433. https://doi.org/10.1079/IVP2004541

Farrokhi, E., Koocheki, A., Mahallati, M. N., & Khademi, R. (2017). *The Effect of Manure, Chemical and Biological Fertilizers on Aloe vera Growth in Boushehr Province.* https://www.google.com

Fay, M. F. (1994). In what situations is in vitro culture appropriate to plant conservations? *Biodiversity and Conservation*, *3*(2), 176–183. https://doi.org/10.1007/BF02291887

Foden, W. B. (2002). *A Demographic Study of Aloe dichotoma in the Succulent Karoo: Are the Effects of Climate Change Already Apparent?* University of Cape Town. https://www.google.com

Foden, W., Midgley, G. F., Hughes, G., Bond, W. J., Thuiller, W., Hoffman, M. T., Kaleme, P., Underhill, L. G., Rebelo, A., & Hannah, L. (2007). A changing climate is eroding the geographical range of the Namib Desert tree *Aloe* through population declines and dispersal lags. *Diversity and Distributions*, *13*(5), 645–653. https://doi.org/10.1111/j.1472-4642.2007.00391.x

Forbes, R. W., Craig, A. J. F. K., Hulley, P. E., & Parker, D. M. (2009). Seasonal variation in the avian community associated with an *Aloe ferox* (*Asphodelaceae*, Mill .) flowering event in the Eastern Cape, South Africa. *Proceedings of the 12th Pan-African Ornithological Congress, 2008*, *Cotton 2001*. https://www.researchgate.net/publication/288559170

Furer, V., Hersch, M., Silvetzki, N., Breuer, G. S., & Zevin, S. (2011). *Nicotiana glauca* (Tree Tobacco) Intoxication-Two Cases in One Family. *Journal of Medical Toxicology*, *7*(1), 47–51. https://doi.org/10.1007/s13181-010-0102-x

Gantait, S., Sinniah, U. R., & Das, P. K. (2014). *Aloe vera*: A review update on advancement of in vitro culture. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, 64 (1), 1–12. https://doi.org/10.1080/09064710.2013.868924

Genena, A. K., Hense, H., Smânia, A., & De Souza, S. M. (2008). Rosemary (*Rosmarinus officinalis*): A study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. *Ciencia e Tecnologia de Alimentos*, *28*(2), 463–469. https://doi.org/10.1590/S0101-20612008000200030

Giddy, C. (1973). Aloes from Seed. *Journal of the Botanical Society of South Africa*, *59*, 41–44. https://hdl.handle.net/10520/AJA00423203_4070

Godoy, F. L., Tabor, K., Burgess, N. D., Mbilinyi, B. P., Kashaigili, J. J., & Steininger, M. K. (2011). Deforestation and CO_2 emissions in coastal Tanzania from 1990 to 2007. *Environmental Conservation*, *39*(1), 62–71. https://doi.org/10.1017/S037689291100035X

Grace, O. M. (2011). Current perspectives on the economic botany of the genus *Aloe* L. (*Xanthorrhoeaceae*). *South African Journal of Botany*, *77*(4), 980–987. https:// doi. org/ 10. 1016/j. sajb.2011.07.002

Grace, O. M., Simmonds, M. S. J., Smith, G. F., & Van Wyk, A. E. (2008). Therapeutic uses of *Aloe* L. (*Asphodelaceae*) in southern Africa. *Journal of Ethnopharmacology, 119*(3), 604–614. https://doi.org/10.1016/j.jep.2008.07.002

Grace, O. M., Buerki, S., Symonds, M. R. E., Forest, F., Van Wyk, A. E., Smith, G. F., Klopper, R. R., Bjorå, C. S., Neale, S., Demissew, S., Simmonds, M. S. J., & Rønsted, N. (2015). Evolutionary history and leaf succulence as explanations for medicinal use in aloes and the global popularity of *Aloe vera*. *BMC Evolutionary Biology, 15*(1), 1–12. https://doi.org/10.1186/s12862-015-0291-7

Grace, O. M., Simmonds, M. S. J., Smith, G. F., & Van Wyk, A. E. (2009). Documented utility and biocultural value of *Aloe* L. (*Asphodelaceae*): A review. *Economic Botany*, *63*(2), 167–178. https://doi.org/10.1007/s12231-009-9082-7

Groenewald, E. G., Koeleman, A., & Wessels, D. C. J. (1975). Callus Formation and Plant Regeneration From Seed Tissue of *Aloe pretoriensis* Pole Evans. *Zeitschrift Für Pflanzenphysiologie*, *75*(3), 270–272. https://doi.org/10.1016/s0044-328x(75)80089-4

Gupta, S. (2014). In-vitro Propagation of *Aloe vera* (L.) Burm. f. *British Biotechnology Journal*, *4*(7), 806–816. https://doi.org/10.9734/bbj/2014/9747

Hall, J., Burgess, N. D., Lovett, J., Mbilinyi, B., & Gereau, R. E. (2009). Conservation implications of deforestation across an elevational gradient in the Eastern Arc Mountains, Tanzania. *Biological Conservation*, *142*(11), 2510–2521. https:// doi. org/ 10. 1016/j. biocon. 2009.05.028

Haller, J. S. (1990). A drug for all seasons. Medical and pharmacological history of aloe. *Bulletin of the New York Academy of Medicine*, *66*(6), 647–659. https:// www. researchgate. net/ publication/ 20874527

Hamasha, H. R., & Hensen, I. (2009). Seed germination of four *Jordanian Stipa* spp: Differences in temperature regimes and seed provenances. *Plant Species Biology*, *24*(2), 127–132. https://doi.org/10.1111/j.1442-1984.2009.00247.x

Hankey, A., & Smith, G. F. (2006). Notes on the cultivation and propagation of *Chortolirion angolense* (Baker) A. Berger (*Asphodelaceae*). *Bradleya*, *24*(24), 89–92. https:// doi. org/ 10. 25223/ brad. n24.2006.a9

Hasanuzzaman, M., Bhuyan, M. H. M. B., Nahar, K., Hossain, M. S., Al Mahmud, J., Hossen, M. S., Masud, A. A. C., & Fujita, M. (2018). Potassium: A vital regulator of plant responses and tolerance to abiotic stresses. *Agronomy*, *8*(3), 2–29. https:// doi. org/ 10. 3390/ agronomy8030031

Howell, K. M., Burgess, N. D., Butynski, T. M., Cordeiro, N. J., Doggart, N. H., Fjeldsa, J., Kilahama, F. B., Loader, S. P., Lovett, J. C., Mbilinyi, B., Menegon, M., Moyer, D. C., Nashanda, E., Perkin, A., Rovero, F., Stanley, W. T., & Stuart, S. N. (2006). The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. *Biological Conservation*, *134*(2), 209–231. https://doi.org/10.1016/j.biocon.2006.08.015

Huebner, C. D. (2007). Detection and Monitoring of Invasive Exotic Plants: A Comparison of Four Sampling Methods. *Northeastern Naturalist*, *14*(2), 183–206. https:// doi. org/ 10. 1656/ 1092-6194(2007)14[183:damoie]2.0.co;2

Isah, T. (2018). Secondary metabolism of pharmaceuticals in the plant in vitro cultures: Strategies, approaches, and limitations to achieving higher yield. *Plant Cell, Tissue and Organ Culture*, *132*(2), 239–265. https://doi.org/10.1007/s11240-017-1332-2

IUCN. (2011). *Guidelines for appropriate uses of IUCN Red List Data. Incorporating the Guidelines for Reporting on Proportion Threatened and the Guidelines on Scientific Collecting of Threatened Species.* The IUCN Red List Committee and IUCN SSC Steering Committee. http://www.iucnredlist.org/documents/RL_Guidelines_Data_Use.pdf

IUCN Standards and Petitions Committee. (2019). IUCN Standards and Petitions Committee. *Standards and Petitions Committee*. https:// doi. org/ http:// www. iucnredlist. org/ documents/ RedListGuidelines.pdf.

IUCN Standards and Petitions Subcommittee. (2014). *Guidelines for Using the IUCN Red List Categories and Criteria the iucn red list of threatened species*. http:// www. iucnredlist. org/ documents/ RedListGuidelines. pdf.%0Ahttp:// www. iucnredlist. org/ documents/ RedListGuidelines.pdf

Jain, R., Sinha, A., Kachhwaha, S., & Kothari, S. L. (2009). Micropropagation of withania coagulans (stocks) dunal: A critically endangered medicinal herb. *Journal of Plant Biochemistry and Biotechnology*, *18*(2), 249–252. https://doi.org/10.1007/BF03263330

Jin, Z. M., Wang, C. H., Liu, Z. P., & Gong, W. J. (2007). Physiological and ecological characters studies on *Aloe vera* under soil salinity and seawater irrigation. *Process Biochemistry*, *42*(4), 710–714. https://doi.org/10.1016/j.procbio.2006.11.002

Kader, M. A. (2005). A Comparison of Seed Germination Calculation Formulae and the Associated Interpretation of Resulting Data. *Journal and Proceeding of the Royal Society of New South Wales*, *138*, 65–75. https://royalsoc.org.au/images/pdf/journal/138_Kader.pdf

Kahn, A. E., & Durako, M. J. (2005). The effect of salinity and ammonium on seed germination in *Ruppia maritima* from Florida Bay. *Bulletin of Marine Science*, *77*(3), 453–458. https://www.researchgate.net/publication/239558833

Kaviani, B., & Branch, R. (2015). Some useful information about micropropagation. *Journal of Ornamental Plants*, *5*(1), 29–40. http:// jornamental. iaurasht. ac .ir/article_ 513288_ 2933de76ea7932b748d431cf616b0700.pdf

Kawai, K., Beppu, H., Koike, T., Fujita, K., & Marunouchi, T. (1993). Tissue culture of *Aloe arborescens* Miller var. *natalensis* Berger. *Phytotherapy Research*, *7*(7), S5–S10. https://doi.org/10.1002/ptr.2650070705

Kaya, C., Tuna, A. L., Ashraf, M., & Altunlu, H. (2007). Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. *Environmental and Experimental Botany*, *60*(3), 397–403. https://doi.org/10.1016/j.envexpbot.2006.12.008

Kazuhiko, H., Wajirou, S., & Satohiko, S. (1997). Impact of secondary seed dispersal and herbivory on seedling survival in *Aesculus turbinata*. *Journal of Vegetatation Science, 8*, 735–742. https://doi.org/10.2307/3237379

Kidane, B., Van Andel, T., Van der Maesen, L. J. G., & Asfaw, Z. (2014). Use and management of traditional medicinal plants by Maale and Ari ethnic communities in southern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *10*(1), 1–15. https://doi.org/10.1186/1746-4269-10-46

Kideghesho, J. R., & Msuya, T. S. (2010). Gender and socio-economic factors influencing domestication of indigenous medicinal plants in the West Usambara Mountains, northern Tanzania. *International Journal of Biodiversity Science, Ecosystem Services and Management*, *6*(1–2), 3–12. https://doi.org/10.1080/17451590.2010.480946

Kiran, S., Tirkey, A., Jha, Z., & Porte, S. S. (2017). In–Vitro Regeneration of *Aloe vera* (*Aloe barbadensis* Mill). *International Journal of Current Microbiology and Applied Sciences*, *6*(11), 1829–1834. https://doi.org/10.20546/ijcmas.2017.611.218

Kleyheeg, E., Claessens, M., & Soons, M. B. (2018). Interactions between seed traits and digestive processes determine the germinability of bird-dispersed seeds. *PLoS One*, *13*(4), 1–15. https://doi.org/10.1371/journal.pone.0195026

Klopper, R. R., Smith, G. F., Crouch, N. R., Grace, O. M., & Demissew, S. (2013). Aloes of the world project: Background and progress. *Scripta Botanica Belgica*, *50*, 332–336. https://www.researchgate.net/publication/256628682

Knapp, A. (2006). A review of the trade in *Aloe ferox*, with a focus on the role of the European Union. *A TRAFFIC Europe Report for the European Commission, Brussels, and Belgium*. https://www.google.com

Kulkarni, M. G., Sparg, S. G., & Van Staden, J. (2006). Dark conditioning, cold stratification and a smoke-derived compound enhance the germination of *Eucomis autumnalis* subsp. *autumnalis* seeds. *South African Journal of Botany*, *72*(1), 157–162. https:// doi. org/ 10. 1016/j. sajb. 2005.06.006

Kulkarni, M. G., Amoo, S. O., Kandari, L. S., & Van Staden, J. (2013). Seed germination and phytochemical evaluation in seedlings of *Aloe arborescens* Mill. *Plant Biosystems, 148*(3), 460–466. https://doi.org/10.1080/11263504.2013.782901

Kutnar, L., & Hladnik, D. (2016). Forest Ecology and Management Assessing biodiversity and conservation status of the Natura 2000 forest habitat types: Tools for designated forestlands stewardship. *Forest Ecology and Management*, *359*, 256–267. https:// doi. org/10. 1016/j. foreco. 2015.10.011

Larson, D. W., Matthes, U., & Kelly, P. E. (2005). *Cliff Ecology: Pattern and Process in Cliff Ecosystems*. Cambridge University Press. New York, US. https://www.google.com

Le Stradic, S., Silveira, F. A. O., Buisson, E., Cazelles, K., Carvalho, V., & Fernandes, G. W. (2015). Diversity of germination strategies and seed dormancy in herbaceous species of *Campo rupestre* grasslands. *Austral Ecology, 40*(5), 537–546. https:// doi. org/ 10. 1111/ aec. 12221

Letsara, R., Rakotoarisoa, S., & Almeda, F. (2012). Three new *Aloe* species from Madagascar. *Malagasy Nature, 6*(1), 46–55. https://www.researchgate.net/publication/236178106

Liao, Z., Chen, M., Tan, F., Sun, X., & Tang, K. (2004). Microprogagation of endangered Chinese aloe. *Plant Cell, Tissue and Organ Culture*, *76*(1), 83–86. https:// doi. org/ 10. 1023/A:1025868515705

Liu, H. M., Xu, Z. F., Xu, Y. K., & Wamg, J. H. (2002). Practice of conservation plant diversity through traditional beliefs: A case study in the Xishuangbanna, southeast China. *Biodiversity and Conservation, 11 SRC*, 705–713. http://dx.doi.org/10.1023/A:1015532230442

Liu, X., Li, J., Zhang, Y., Li, L., & He, D. (2011). Biological research advancement in *Aloe*. *Journal of Medicinal Plants Research, 5*(7), 1046–1052. https:// doi. org/ 10. 5897/JMPR. 9000307

Lobo, J. M., & Chefaoui, R. M. (2005). Potential distribution modelling, niche characterization and conservation status assessment using GIS tools: A case study of Iberian *Copris* species.

Biological Conservation, *122*(2), 327–338. https://doi.org/10.1016/j.biocon.2004.08.005

Luhunga, P. M., Kijazi, A. L., Chang'a, L., Kondowe, A., Ng'ongolo, H., & Mtongori, H. (2018). Climate change projections for Tanzania Based on high-resolution regional climate models from the Coordinated Regional Climate Downscaling Experiment (CORDEX)-Africa. *Frontiers in Environmental Science*, *6*, 1–20. https://doi.org/10.3389/fenvs.2018.00122

Magoro, M. D., Masoga, M. A., & Mearns, M. A. (2010). Traditional health practitioners' practices and the sustainability of extinction-prone traditional medicinal plants. *International Journal of African Renaissance Studies-Multi-Inter-and Transdisciplinarity*, *5*(2), 229–241. https://doi.org/10.1080/18186874.2010.534842

Majamba, H. (2000). *"Implementation and Enforcement of the Convention on International Trade in Endangered Species of Wild Fauna and Flora in Tanzania". Uganda Wildlife Society Research Report No. 2*. https://www.academia.edu/13123775

Manvitha, K., & Bidya, B. (2014). *Aloe vera*: A wonder plant its history, cultivation and medicinal uses. *Journal of Pharmacognosy and Phytochemistry*, *2*(25), 85–88. http:// www. phytojournal.com/vol2Issue5/Issue_jan_2014/19.1.pdf

Markham, J. (2014). Rare species occupy uncommon niches. *Scientific Reports*, *4*(4), 63–65. https://doi.org/10.1038/srep06012

Mazri, M. (2012). Effect of Liquid Media and In Vitro Pre-acclimatization Stage on Shoot Elongation and Acclimatization of Date Palm (*Phoenix dactylifera* L.) cv. Najda. *Journal of Ornamental and Horticultural Plants*, *2*(4), 225–231. https:// www. researchgate. net/ publication/ 265927648

McCaffrey, N. B., Blick, R. A. J., Glenn, V. C., Fletcher, A. T., Erskine, P. D., & Van Osta, J. (2014). Novel "stratified-meander" technique improves survey effort of the rare Pagoda Rock Daisy growing remotely on rocky cliff edges. *Ecological Management and Restoration*, *15*(1), 94–97. https://doi.org/10.1111/emr.12087

Mikulík, J. (1999). Propagation of endangered plant species by tissue cultures. *In Vitro*, *1*(37), 27–33. https://www.researchgate.net/publication/265927648

Moghbeli, E. (2012). Effects of salinity stress on growth and yield of *Aloe vera* L. *Journal of Medicinal Plants Research*, *6*(16), 3272–3277. https://doi.org/10.5897/jmpr11.1698

Molsaghi, M., Moieni, A., Kahrizi, D., Molsaghi, M., Moieni, A., & Kahrizi, D. (2014). Efficient protocol for rapid *Aloe vera* micropropagation. *Pharmaceutical Biology*, *52*(6), 735–739. https://doi.org/10.3109/13880209.2013.868494

Msogoya, T., Kanyagha, H., Mutigitu, J., Kulebelwa, M., & Mamiro, D. (2012). Identification and management of microbial contaminants of banana in vitro cultures. *Jornal of Applied Biosciences*, *55*, 33987–33994. https://www.researchgate.net/publication/235722908

Murashige, T. (1974). Plant Propagation Through Tissue Cultures. *Annual Review of Plant Physiology*, *25*(1), 135–166. https://doi.org/10.1146/annurev.pp.25.060174.001031

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio-assays with Tobacco tissue cultures. *Physiologia Plantarumochemistry*, *15*(4), 73–95. https:// doi. org/ 10. 1016/ S0031-9422(01)00179-0

Murillo-Amador, B., Córdoba-Matson, M. V., Villegas-Espinoza, J. A., Hernández-Montiel, L. G., Troyo-Diéguez, E., & García-Hernández, J. L. (2014). Mineral content and biochemical variables of *Aloe vera* L. under salt stress. *PLoS ONE*, *9*(4), e94870. https:// doi. org/ 10. 1371/ journal.pone.0094870

Murray, B. R., Thrall, P. H., Gill, A. M., & Nicotra, A. B. (2002). How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology*, *27*(3), 291–310. https://doi.org/10.1046/j.1442-9993.2002.01181.x

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*(6772), 853–858. http:// dx. doi. org/ 10.1038/35002501

Natali, L., Sanchez, I. C., & Cavallini, A. (1990). In vitro culture of *Aloe barbadensis* Mill.: Micropropagation from vegetative meristems. *Plant Cell, Tissue and Organ Culture*, *20*(1), 71–74. https://doi.org/10.1007/BF00034761

Nayanakantha, N., Singh, B., & Kumar, A. (2011). Improved culture medium for micropropagation of *Aloe vera* L. *Tropical Agricultural Research and Extension*, *13*(4), 87. https://doi.org/10.4038/tare.v13i4.3291

Ndhlala, A. R., Ncube, B., Okem, A., Mulaudzi, R. B., & Van Staden, J. (2013). Toxicology of some important medicinal plants in southern Africa. *Food and Chemical Toxicology*, *62*(1), 609–621. https://doi.org/10.1016/j.fct.2013.09.027

Newmark, W. (2002). *Conserving Biodiversity in East African Forest: A study of the Eastern Arc Mountains*. Springer-Verlag Berlin Heidelberg. http://dx.doi.org/10.1007/978-3-662-04872-6

Oredsson, A. (1997). Threatened species not necessarily rare, rare species not necessarily threatened. *Environmental Conservation*, *24*(3), 207–209. https:// doi. org/ 10. 1017/ S0376892997000283

Otieno, J., Abihudi, S., Veldman, S., Nahashon, M., Van Andel, T., & De Boer, H. J. (2015). Vernacular dominance in folk taxonomy: A case study of ethnospecies in medicinal plant trade in Tanzania. *Journal of Ethnobiology and Ethnomedicine*, *11*(1), 1-10. https:// doi. org/ 10. 1186/1746-4269-11-10

Ouborg, N. J., Vergeer, P., & Mix, C. (2006). The rough edges of the conservation genetics paradigm for plants. *Journal of Ecology*, 94(6), 1233–1248). https://doi.org/10.1111/j.1365-2745.2006.01167.x

Pande, S. S., & Gupta, P. (2013). Plant tissue culture of *Stevia rebaudiana* (Bertoni): A review. *Journal of Pharmacognosy and Phytotherapy*, *5*(1), 26–33. https:// doi. org/ 10. 5897/JPP13.

Pandey, R., & Mishra, A. (2010). Antibacterial activities of crude extract of *Aloe barbadensis* to clinically isolated bacterial pathogens. *Applied Biochemistry and Biotechnology*, *160*(5), 1356–1361. https://doi.org/10.1007/s12010-009-8577-0

Pascaline, J., Charles, M., George, O., Lukhoba, C., & Ruth, L. (2010). Ethnobotanical survey and propagation of some endangered medicinal plants from south Nandi district of Kenya. *Journal of Animal & Plant Sciences*, *8*(3), 1016–1043. https:// www. researchgate. net/ publication/ 290488064

Patil, A., Thakur, D., Govill, K., Kumar, D., & Yadav, P. K. (2017). *Aloe vera* as potential emerging herbal feed additive: A boon for livestock rearing. *International Journal of Chemical Studies*, *5*(54), 494–502. https://www.researchgate.net/publication/318393878

Patric, M., Peris, K., & Oscar, E. M. (2006). *Threats to Medicinal Plant Species-an African Perspective. A Global Synthesis Work Shop on Biodiversity Loss and Species Extinctions: Managing Risk in a Changing World*. Ecosystems and Livelihoods Group, Asia. Asian Regional Office. The World Conservation Union (IUCN). https:// doi. org/ http:// books.

google. co.ke/ books? id=QKd6qumD -rwC&pg= PR3&lpg= PR3&dq= threats+ to+ medicinal+plants,+an+african+perspective,+maundu,+Kariuki,+IUCN&source=bl&ots=hYhV7OqXwW&sig=cCTdMj97baN34RjCUCJfL_LrZCg&hl=en&ei=0oukSpG1L9HfnAeZ5NyFBQ&sa=X&oi=book_result&ct=re

Payne, S. L., Witkowski, E. T. F., & Symes, C. T. (2019). Good times, bad times: Inter-annual reproductive output in a montane endemic succulent (*Aloe peglerae*; *Asphodelaceae*) driven by contrasting visitor responses of small mammals and birds. *Austalian Journal of Botany*, *67*, 116–127. http://dx.doi.org/10.1071/BT18163

Pfab, M. F., & Victor, J. E. (2002). Threatened plants of Gauteng, South Africa. *South African Journal of Botany*, *68*(3), 370–375. https://doi.org/10.1016/S0254-6299(15)30400-2

Pfab, M. F, & Witkowski, E. T. F. (1999). Fire survival of the Critically Endangered succulent, *Euphorbia clivicola* R. A. Dyer: Fire-avoider or fire-tolerant? *African Journal of Ecology*, *37*(3), 249–257. https://doi.org/10.1046/j.1365-2028.1999.00176.x

Pfab, M. F., & Scholes, M. A. (2004). Is the collection of *Aloe peglerae* from the wild sustainable? An evaluation using stochastic population modelling. *Biological Conservation*, *118*(5), 695–701. https://doi.org/10.1016/j.biocon.2003.10.018

Phama, J. O., Panagos, M. D., Myburgh, W. J., & Pfab, M. F. (2014). The population status of the Endangered endemic plant *Aloe peglerae*: Area of occupancy, population structure, and past population trends. *South African Journal of Botany*, *93*, 247–251. https:// doi. org/ 10. 1016/j. sajb.2014.04.015

Pili, K. (2007). Aloe: Beyond Use As Cosmetics. *Dar Es Salaam Medical Students' Journal*, *15*, 31–35. https://doi.org/10.4314/dmsj.v15i1.52495

Radha, M. H., & Laxmipriya, N. P. (2015). Evaluation of biological properties and clinical effectiveness of *Aloe vera*: A systematic review. *Journal of Traditional and Complementary Medicine*, *5*(1), 21–26. https://doi.org/10.1016/j.jtcme.2014.10.006

Rai, M., Rathod, D., Agarkar, G., Dar, M., Brestic, M., Pastore, G. M., & Junior, M. R. M. (2014). Fungal growth promotor endophytes: A pragmatic approach towards sustainable food and agriculture. *Symbiosis*, *62*(2), 63–79. https://doi.org/10.1007/s13199-014-0273-3

Rajamanikyam, M., Vadlapudi, V., Amanchy, R., & Upadhyayula, S. M. (2017). Endophytic Fungi as Novel Resources of Natural Therapeutics. *Archives of Biology and Technology*, 12, 1–26. http://dx.doi.org/10.1590/1678-4324-2017160542

Reed, B. M., & Tanprasert, P. (1995). Detection and control of bacterial contaminants of plant tissue cultures: A review of recent literature. *Plant Tissue Culture and Biotechnology*, *1*(3), 137–142. https://www.researchgate.net/publication/222714440

Renu, T., Jyoti, G., Sheikh, A., Rajneesh, P., & Rashmi, H. (2011). *Aloe vera* and its uses in Dentistry. *Indian Journal of Dental Advancement*, *3*(4), 656–658. https:// doi. org/ 10. 5866/ 3.4.656

Reynolds, T. (2004a). *Aloe: The genus Aloe.* DC. CRC press. Washington, D.C. https:// doi. org/ 10. 1016/j.phymed.2007.11.006

Reynolds, T. (2004b). *Aloes in habitat*. CRC Press. Washington, D.C. https://www.google.com

Reynolds, T. (2004c). *Analytical methodology: The exudate*. CRC Press. Washington, D.C. https://www.google.com

Richart, M. (2019a). *Aloe duckeri*. The IUCN Red List of Threatened Species 2019: e. T110717100A110717103. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2019- 3. RLTS. T110717100A110717103.en.

Richart, M. (2019b). *Aloe myriacantha*, Grass Aloe. The IUCN Red List of Threatened Species 2019: e. T110763474A110763500. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2019- 3. RLTS. T110763474A110763500.en Copyright.

Richart, M. (2019c). *Aloe nuttii*. In The IUCN Red List of Threatened Species 2019: e. T110765906A110765909. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2019- 3. RLTS. T110765906A110765909.en.

Rowntree, J. K., Pressel, S., Ramsay, M. M., Sabovljevic, A., & Sabovljevic, M. (2011). In vitro conservation of *European bryophytes*. *In Vitro Cellular and Developmental Biology - Plant*, 47(1), 55–64. https://doi.org/10.1007/s11627-010-9326-3

Roy, S. C., & Sarkar, A. (1991). In vitro regeneration and micropropagation of *Aloe vera* L. *Scientia*. *Horticulturae*, *47*(1–2), 107–113. https://doi.org/10.1016/0304-4238(91)90032-T

Sachedina, H., & Bodeker, G. (1999). Wild Aloe harvesting in South Africa. *Journal of Alternative* *and Complementary Medicine*, *5*(2), 121–123. https:// doi. org/ 10. 1089/ acm. 1999. 5.121

Sajem, A. L., Rout, J., & Nath, M. (2008). Traditional Tribal Knowledge and Status of some Rare and Endemic Medicinal Plants of North Cachar Hills District of Assam, Northeast India. *Ethnobotanical Leaflets*, *12*(24), 261–275. https:// www. researchgate. net/ publication/ 41115599

Sarasan, V., Kite, G. C., Sileshi, G. W., & Stevenson, P. C. (2011). Applications of phytochemical and in vitro techniques for reducing over-harvesting of medicinal and pesticidal plants and generating income for the rural poor. *Plant Cell Reports*, 30(7) 1163–1172). https://doi.org/10.1007/s00299-011-1047-5

Sarper, F., Akaydin, G., Şimşek, I., & Yeşilada, E. (2009). An ethnobotanical field survey in the Haymana district of Ankara province in Turkey. *Turkish Journal of Biology*, *33*(1), 79–88. https://doi.org/10.3906/biy-0808-28

Schippmann, U., Cunningham, A. B., Leaman, D. J., & Walter, S. (2005). Impact of cultivation and collection on the conservation of medicinal plants: Global trends and issues. *Acta Horticulturae*, *676*(1), 31–44. https://doi.org/10.17660/actahortic.2005.676.3

Schmelzer, G., Gurib-Fakim, A., Arroo, R., Bosch, C., Ruijter, A., & Simmonds, M. (2008). *Plant Resources of Tropical Africa.* PROTA Foundation, Wageningen/Backhuys. https://doi.org/10.2307/4120640. https://www.google.com

Shahzad, M. N., & Ahmed, N. (2013). Effectiveness of *Aloe vera* gel compared with 1% silver sulphadiazine cream as burn wound dressing in second degree burns. *Journal of the Pakistan Medical Association*, *63*(2), 225–230. https://www.researchgate.net/publication/253336687

Sharma, N., Chandel, K. P. S., & Paul, A. (1993). In vitro propagation of Gentiana kurroo: An indigenous threatened plant of medicinal importance. *Plant Cell, Tissue and Organ Culture*, *34*(3), 307–309. https://doi.org/10.1007/BF00029722

Sharma, S., Rathi, N., Kamal, B., Pundir, D., Kaur, B., & Arya, S. (2010). Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology: A review. *Agriculture and Biology Journal of North America*, *1*(5), 827–833. https://doi.org/10.5251/abjna.2010.1.5.827.833

Shekhawat, N. S., Rathore, M. S., Shekhawat, S., Choudhary, S. K., Phulwaria, M., Harish, R. M.

K., Vibha, J. B., Rathore, N. S., Patel, A. K., & Kataria, V. (2013). Micropropagation of *Aloe vera* for Improvement and Enhanced Productivity. *Climate Change and Plant Abiotic Stress Tolerance*, 2011, 857–880. https://doi.org/10.1002/9783527675265.ch32

Sidhu, Y. (2010). In vitro micropropagation of medicinal plants by tissue culture. *The Plymouth Student Scientist*, *4*(41), 432–449. https://www.researchgate.net/publication/267683689

Smith, G. F., & Correia, R. I. D. S. (1992). Establishment of *Aloe greatheadii* var. *davyana* from seed for use in reclamation trials. *Landscape and Urban Planning*, *23*(1), 47–54. https://doi.org/10.1016/0169-2046(92)90063-6

Smith, G. F., Klopper, R. R., & Crouch, N. R. (2008). *Aloe arborescens* (*Asphodelaceae*: *Alooideae*) and CITES. *Haseltonia*, *14*(14), 189–198. https://doi.org/10.2985/1070-0048-14.1.189

Solano, E., & Feria, T. P. (2007). Ecological niche modeling and geographic distribution of the genus *Polianthes* L. (*Agavaceae*) in Mexico: Using niche modeling to improve assessments of risk status. *Biodiversity and Conservation*, *16*(6), 1885–1900. https:// doi. org/ 10. 1007/ s10531-006-9091-0

Sõukand, R., & Kalle, R. (2011). Change in medical plant use in Estonian ethnomedicine: A historical comparison between 1888 and 1994. *Journal of Ethnopharmacology*, *135*(2), 251–260. https://doi.org/10.1016/j.jep.2011.02.030

Srithi, K., Balslev, H., Wangpakapattanawong, P., Srisanga, P., & Trisonthi, C. (2009). Medicinal plant knowledge and its erosion among the Mien (Yao) in northern Thailand. *Journal of Ethnopharmacology*, *123*(2), 335–342. https://doi.org/10.1016/j.jep.2009.02.035

Status Reports for 7 Coastal Forests in Tanga Region. (1989). *Kilulu hill forest*. https://www.google.com

Staub, P. O., Geck, M. S., Weckerle, C. S., Casu, L., & Leonti, M. (2015). Classifying diseases and remedies in ethnomedicine and ethnopharmacology. *Journal of Ethnopharmacology*, *174*, 514–519. https://doi.org/10.1016/j.jep.2015.08.051

Stokes, C. J., & Yeaton, R. I. (1995). Population dynamics, pollination ecology and the significance of plant height in *Aloe candelabrum*. *African Journal of Ecology*, *33*(2), 101–113. https://doi.org/10.1111/j.1365-2028.1995.tb00786.x

Sultan, P., Shawl, A. S., Ramteke, P. W., Jan, A., Chisti, N., Jabeen, N., & Shabir, S. (2006). In vitro propagation for mass multiplication of *Podophyllum hexandrum*: A high value medicinal herb. *Asian Journal of Plant Sciences*, *5*(2), 179–184. https:// doi. org/ 10. 3923/ ajps. 2006.179.184

Surjushe, A., Vasani, R., & Saple, D. G. (2008). *Aloe vera*: A short review. *Indian Journal of Dermatology*, *53*(4), 163–168. https://doi.org/10.4103/0019-5154.44785

Symes, C. T. (2012). Seed dispersal and seed banks in *Aloe marlothii* (*Asphodelaceae*). *South African Journal of Botany*, *78*, 276–280. https://doi.org/10.1016/j.sajb.2011.06.008

Symes, C. T. (2017). Notes on the flowering and pollination of the endemic grassland *Aloe reitzii* var. *reitzii* (*Asphodelaceae*). *Bothalia*, *47*(1), 5–9. https:// doi. org/ 10. 4102/ abc. v47i1. 2215

Tabor, K., Kashaigili, J. J., Mbilinyi, B., & Wright, T. M. (2010). *Forest cover and change for the Eastern Arc Mountains and Coastal Forests of Tanzania and Kenya circa 2000 to circa 2010. Final report*. https://www.google.com

Teketay, D. (1996). The effect of different pre-sowing seed treatments, temperature and light on the germination of five *Senna* species from Ethiopia. *New Forests*, *11*(2), 155–171. https://doi.org/10.1007/BF00033411

Thuiller, W., Albert, C., Araújo, M. B., Berry, P. M., Cabeza, M., Guisan, A., Hickler, T., Midgley, G. F., Paterson, J., Schurr, F. M., Sykes, M. T., & Zimmermann, N. E. (2008). Predicting global change impacts on plant species' distributions: Future challenges. *Perspectives in Plant Ecology, Evolution and Systematics*, *9*(3–4), 137–152. https:// doi. org/ 10. 1016/ j. ppees. 2007. 09.004

Torres-Avilez, W., Medeiros, P. M., & Albuquerque, U. P. (2016). Effect of Gender on the Knowledge of Medicinal Plants: Systematic Review and Meta-Analysis. *Evidence-Based Complementary and Alternative Medicine*, *2016*, 1–13. https:// doi. org/ 10. 1155/ 2016/ 6592363

Traveset, A., Riera, N., & Mas, R. E. (2001). Passage through bird guts causes interspecific differences. *Functional Ecology*, *15*, 669–675. https:// doi. org/ 10. 1046/j. 0269-8463. 2001. 00561.x

Trivedi, N., Rishi, P., & Soni, S. K. (2012). Production of a herbal wine from *Aloe vera* gel and evaluation of its effect against common food borne pathogens and probiotics. *International Journal of Food and Fermentation Technology, 2*(2), 157–166. https:// www. researchgate. net/publication/325809641

UNEP. (1995). *Environmenr information System Development in the SADC Region*. https://www.google.com

Upadhyay, R., & Mishra, H. N. (2015). Multivariate Analysis for Kinetic Modeling of Oxidative Stability and Shelf Life Estimation of Sunflower Oil Blended with Sage (*Salvia officinalis*) Extract Under Rancimat Conditions. *Food and Bioprocess Technology*, *8*(4), 801–810. https://doi.org/10.1007/s11947-014-1446-z

Van der Merwe, D., Swan, G. E., & Botha, C. J. (2001). Use of ethnoveterinary medicinal plants in cattle by Setswana-speaking people in the Madikwe area of the North West Province of South Africa. *Journal of the South African Veterinary Association*, *72*(4), 189–196. https://doi.org/10.4102/jsava.v72i4.651

Van Hoang, S., Baas, P., & Keßler, P. J. A. (2008). Uses and conservation of plant species in a national park: A case study of Ben En, Vietnam. *Economic Botany*, *62*(4), 574–593. https://doi.org/10.1007/s12231-008-9056-1

Van Wyk, B. E., & Smith, G. (1996). *Guide to the aloes of South Africa*. Briza Publishers. https://www.google.com

Vázquez-Yanes, C., & Orozco-Segovia, A. (1993). Patterns of Seed Longevity and Germination in the Tropical Rainforest. *Annual Review of Ecology and Systematics*, *24*(1), 69–87. https://doi.org/10.1146/annurev.es.24.110193.000441

Verma, S. (2011). *Aloe vera* their chemicals composition and applications: A review. *International Journal of Biological & Medical Research*, *2*(1), 466–471. https:// www. researchgate. net/ publication/ 48347118

Wabuyele, E. (2006). *Studies on East African Aloe Species: Aspects of Taxonomy, Conservation and Ethnobotany*. University of Oslo, Norway. https://www.google.com

Wabuyele, E., Bjorå, C. S., Nordal, I., & Newton, L. E. (2006). Distribution, Diversity and Conservation of the Genus *Aloe* in Kenya. *Journal of East African Natural History*, *95*(2), 213–225. https://doi.org/10.2982/0012-8317(2006)95[213:ddacot]2.0.co;2

Wabuyele, E., & Kyalo, S. (2008). WG 3 - Succulents and Cycads. Case Study 1 - Sustainable use of East African Aloes: The case of commercial Aloes in Kenya. *International Expert Workshop on CITES Non-Detrimental Findings*. https://www.google.com

Wabuyele, E., Kyalo, S., & Africa, E. (2008). *Sustainable Use of East African Aloes: The Case of commercial Aloes in Kenya*. https://www.researchgate.net/publication/237728629

Weber, A. (2013). *Aloe lateritia*. The IUCN Red List of Threatened Species 2013: e.T201337A2700528. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2013-2. RLTS. T201337A2700528.en.

Weber, A., & Demissew, S. (2013a). *Aloe parvidens*. The IUCN Red List of Threatened Species 2013: e. T201328A2699860. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2013- 2. RLTS. T201328A2699860.en.

Weber, A., & Demissew, S. (2013b). *Aloe secundiflora*. The IUCN Red List of Threatened Species 2013: e. T201405A2705516. http:// dx. doi. org/ 10. 2305/ IUCN. UK. 2013- 2. RLTS. T201405A2705516.en.

Weckerle, C. S., De Boer, H. J., Puri, R. K., Van Andel, T., Bussmann, R. W., & Leonti, M. (2018). Recommended standards for conducting and reporting ethnopharmacological field studies. *Journal of Ethnopharmacology*, *210*, 125–132. https:// doi. org/ 10. 1016/j. jep. 2017. 08.018

Wilfred, P., Milner-Gulland, E. J., & Travers, H. (2019). Attitudes to illegal behaviour and conservation in western Tanzania. *ORYX*, *53*(3), 513–522. https:// doi. org/ 10. 1017/ S0030605317000862

Willis, A. J., & Chapman, V. J. (2006). Coastal Vegetation. *The Journal of Ecology*, *54*(1), 275. https://doi.org/10.2307/2257673

Wilson, A. L., Ward, D., Brown, M., & Johnson, S. D. (2009). Seed production in a threatened *Aloe* is not affected by bird exclusion or population size. *Plant Ecology*, *203*(2), 173–182. https://doi.org/10.1007/s11258-008-9524-9

Winowiecki, L., Vågen, T. G., Massawe, B., Jelinski, N. A., Lyamchai, C., Sayula, G., & Msoka, E. (2016). Landscape-scale variability of soil health indicators: Effects of cultivation on soil organic carbon in the Usambara Mountains of Tanzania. *Nutrient Cycling in Agroecosystems*, *105*(3), 263–274. https://doi.org/10.1007/s10705-015-9750-1

Wiseman, R. (2001). Woody vegetation change in response to browsing in Ithala Game Reserve, South Africa. *Journal of Wildlife Research*, *34*, 25–37. https:// www. researchgate. net/ publication/33794387

Wochok, Z. S. (1981). The role of tissue culture in preserving threatened and endangered plant species. *Biological Conservation*, *20*(2), 83–89. https:// doi. org/ 10. 1016/ 0006-3207(81)90019-7

Yan, H., Feng, L., Zhao, Y., Feng, L., Wu, D., & Zhu, C. (2020). Prediction of the spatial distribution of *Alternanthera philoxeroides* in China based on ArcGIS and MaxEnt. *Global Ecology and Conservation*, *21*, 1–15. https://doi.org/10.1016/j.gecco.2019.e00856

Yebpella, G. G., Adeyemi, H. M. M., Hammuel, C., Magomya, A. M., Agbaji, A. S., & Okonkwo, E. M. (2011). Phtyochemical screening and comparative study of antimicrobial activity of *Aloe vera* various extracts. *African Journal of Microbiology Research*, *5*(10), 1182–1187. https://doi.org/10.5897/AJMR10.818

Yousafzai, A., Saleem, S., Jahan, N., Javed, F., & Khan, M. W. (2012). *Extraction of Active Components of Aloe vera to Treat Acne / pimple in Population of Quetta City*. https://www.google.com

Zakia, S., Zahid, N. Y., Yaseen, M., Abbasi, N. A., Hafiz, A. A., & Mahmood, N. (2013). Standardization of micropropgation techniques for *Aloe vera*: A pharmaceutically important plant. *Pakistan Journal of Pharmaceutical Sciences*, *26*(6), 1083–1087. https:// www. researchgate. net/publication/258282896

APPENDICES

Appendix 1: Categories for the IUCN Red List

Category	**Explanation**
Extinction (EX)	The species is said to be extinct if there is no reasonable uncertainty that the last individual has died
Extinct in the Wild (EW)	when exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), throughout its historic range have failed to record an individual when it is known only to survive in cultivation, in captivity or as a naturalized population (or populations) well outside the past range
Critically Endangered (CR)	The species is CR if it faces an extremely high risk of extinction in the wild
Endangered (EN)	The species is Endangered if it assessed to be facing a very high risk of extinction in the wild
Vulnerable (VU)	The species is VU if it faces a high risk of extinction in the wild.
Near Threatened (NT)	If the species has been evaluated against the criteria mentioned above but does not qualify. However, it is likely to be eligible for a threatened category soon.
Least Concern (LC)	If the species has been evaluated against the criteria mentioned above but does not qualify. The species is abundant and widespread.
Data Deficient (DD)	There is insufficient data regarding the species to make a primary or secondary assessment of its risk of extinction based on its distribution and/or population status.
Not Evaluated (NE)	A species is Not Evaluated if it has not yet been assessed against the criteria.

Appendix 2: Herbarium voucher specimens (of the numbers SA1-186) and their scientific names, vernacular names of *Aloe* species, and their meaning in English and descriptions as given by respondents (n = 236) in the study area within Tanzania

Scientific name	**Vernacular name**	**Tribe**	**Translation**	**Informant description**
Aloe lateritia Engl. (SA-28, 29, 31, 39, 42,44, 46, 67, 73, 74, 77, 82, 85, 88, 155)	Likulakula	Bena	Aloe	Short spotted Aloe
	Ratuni/Inaboru	Chagga	Aloe	Short Aloe with wide leaves
	Kovongo baridi	Sambaa	Cool Aloe	Spotted and soft, not a true *Aloe vera*.
	Kovongo	Sambaa	Aloe	Spotted Aloe with thin leaves
	Ekighaka/Kighaka	Kurya	Aloe	Spotted Aloe
Aloe rabaiensis Rendle (SA-176-179, 182, 183)	Ratina	Muarusha	Aloe	Spotted Aloe
	Kithapa/Kisapa	Pare	Aloe	Short spotted Aloe
Aloe ballyi Reynolds (SA-172, 180)	Kithapa/Kisapa	Pare	Aloe	Tall Aloe
	Kithapa/Kisapa cha vujawa	Pare	Aloe	Tall Aloe
Aloe bicomitum L.C.Leach (SA-137, 145)	Tembosha/Itemboshya	Fipa	Aloe	Spotted Aloe from the lake side
	Tembosha/Itemboshya	Lungu	Aloe	Sword like Aloe from the lake side
	Tembosha/Itemboshya	Gongwe	Aloe	Green Aloe
Aloe christianii Reynolds	Tembwisha	Pimbwe	Aloe	Green Aloe

Scientific name	Vernacular name	Tribe	Translation	Informant description
(SA-106, 108, 110, 112, 113, 125, 132, 133, 138)	Igaka/Iughaka	Sukuma	Aloe	Green Aloe
	Tembosha/Itemboshya	Nyiha (Mbozi)	Aloe	Green Aloe /unspotted Aloe with sharp spine like sword
	Tembosha/Itemboshya	Fipa	Aloe	Green Aloe
Aloe deserti A.Berger (SA-181)	Kithapa/Kisapa	Kamba	Aloe	Tall Aloe
	Kithapa/Kisapa		Aloe	Short spotted Aloe
Aloe dorotheae A.Berger (SA-47, 48, 49, 69, 76)	Kikoli chekundu	Zigua	Red Aloe	Red with spots
Aloe duckeri Christian (SA-114, 116, 117, 118, 122, 124, 126,128, 129, 131, 134, 140, 142, 148)	Ivata or Amavata (plural)	Nyika (Sumbawanga)	Aloe	Huge spotted Aloe
	Igaka/Lughaka	Sukuma	Aloe	Huge spotted Aloe
	Tembwisha	Pimbwe	Aloe	Spotted and Green Aloe
	Tembosha/Itemboshya	Fipa, Nyika	Aloe	Huge spotted Aloe
Aloe flexilifolia Christian (SA-34, 37)	Kovongo ya majani marefu	Sambaa	Aloe with long leaves	Hanging on cliff surface
	kovongo	Sambaa	Aloe	Aloe with dark leaves
Aloe leptosiphon A.Berger (SA-20, 21, 22, 23, 30, 32, 33, 35, 36, 41)	Katani ya dawa	Sambaa	Medicinal sisal	Spines at the side, bitter taste

Scientific name	Vernacular name	Tribe	Translation	Informant description
	Kovongo kali yamakungu	Sambaa	Bitter Aloe from the cliff	Spots, bitter taste
	Kovongo	Sambaa	Aloe with sports	Used as grease for locally made bicycles
	Kashaza fupi	Sambaa	Short Aloe	Designating ancestor's grave
	Kovongo ya majani mafupi	Sambaa	Aloe with short leaves	Short Aloe
	Katani jamakungu or Katani za kwenye majabali	Sambaa	Aloe from the cliff	Bitter Aloe with spots
	katani ya dawa	Sambaa	Sisal for medicine	Aloe, which turns red in hot areas and green in cold areas
Aloe macrosiphon Baker (SA- 51, 57, 59, 62)	Ekighaka/Kighaka	Kurya	Aloe	Short spotted Aloe, sometimes turns red
Aloe massawana Reynolds (SA-27, 83, 90, 92, 98, 163)	Shubiri Kikoli	Digo Zigua	Aloe	Short spotted Aloe Huge green spotted Aloe
Aloe mzimbana Verd. & Christian (SA-120, 127,130)	Tembosha/Itemboshya	Fipa	Aloe	Small spotted Aloe from the cliff
	Ivata or Amavata (plural)	Nyika	Aloe	Small spotted Aloe from the cliff
	Kinabwele	Pare	Aloe	Spotted Aloe

Scientific name	Vernacular name	Tribe	Translation	Informant description
Aloe parvidens M.G.Gilbert & Sebsebe (SA-171, 174)	Kithapa/Kisapa	Pare	Aloe	Spotted Aloe
	Kinabwele	Pare	Aloe	Spotted Aloe
	Kithapa/Kisapa	Pare	Aloe	Spotted Aloe
Aloe secundiflora Engl. (SA-150, 152, 169, 184)	Olsukuroi miguu mingi	Maasai	Aloe with many legs	Short unspotted Aloe
	Romomoi	Muarusha	Aloe	Short Aloe with wide leaves
	Olsukuroi	Meru	Aloe	Short Aloe with wide leaves
	Olsukuroi	Maasai	Aloe	Short Aloe with wide leaves
	Inaboru	Chaga	Aloe	Short Aloe
	Olsukuroi miguu mingi	Maasai	Aloe with many legs	Short unspotted Aloe
	Romomoi	Muarusha	Aloe	Short Aloe with wide leaves
	Olsukuroi	Meru	Aloe	Short Aloe with wide leaves
	Kinabwele	Pare	Aloe	Unspotted Aloe
	Olsukuroi	Maasai	Aloe	Short Aloe with wide leaves
	Mangoda	Sambaa	Aloe	Tall Aloe

Scientific name	**Vernacular name**	**Tribe**	**Translation**	**Informant description**
Aloe volkensii Engl. (SA-24, 68, 84, 89, 91, 94, 96, 160, 161, 162, 166, 168, 175, 185,186)	Ekighaka/Kighaka	Kurya	Aloe	Tall with spots when young
	Kithapa/Kisapa cha vujawa	Pare	Aloe	Tall Aloe
	Olsukuroi mguu mmoja	Maasai	Aloe with one leg	Tall Aloe
	Kashaza ndefu or Maoza/oza	Sambaa	Tall Aloe	Tall Aloe
	Isale la njovu (tembo)	Chaga	Aloe	Tall Aloe
	Shubiri pori	Digo	Wild Aloe	Tall Aloe
	Likulakula	Bena	Aloe	Tall Aloe
	Ratuni	Chaga	Aloe	Tall Aloe with wide leaves
	Inaboru	Chaga	Aloe	Tall Aloe and short Aloe
Aloe (Unidentified) (SA-45)	Kikoli	Zigua	Aloe	Red/green Aloe
Aloe (Unidentified) (SA-53)	Ekighaka/Kighaka	Kurya	Aloe	Tall Aloe

Appendix 3: Different uses of *Aloe* species in the study areas across Tanzania and the IUCN Red List status of the different *Aloe* species. The IUCN Red List status description: CR=Critically Endangered, EN=Endangered, VU=Vulnerable, and LC=Least Concern

Scientific name	Human ailment	Animal ailment	Cosmetic	Local brew	Others	IUCN Red List Status
A. lateritia	Asthma, Abscess/ boil, Back pain, Bile, Colic, Diabetes, Fungal infection, Gas in the stomach, Heart diseases, Hernia, Blood pressure, Female Infertility, Rushes, Ring worms, Stomachache, Toothache, Wounds, Typhoid	Chicken (Newcastle disease)	Cracks in feet		Removing thorns, Ornamental plant, To remove stains in clothes, Lubricant for wooden tires	LC
A. dorotheae	Skin rushes, Diabetes, Malaria	Chicken (Newcastle disease)				CR
A. flexilifolia	Back pain, Colic, Diabetes, Ear pain, Fainted person, Female infertility, Fungal infection, Headache, Heart diseases, Hernia, HIV, Malaria	Chicken (Newcastle disease)	Dandruff, Cracks in feet		Lubricant for wooden tires	CR
A. massawana	Being numb, Hernia, Malaria, Prevent bleeding, Ringworms, Round worms, Toothache, Ulcers, Wounds	Chicken (Newcastle disease)				VU

Scientific name	Human ailment	Animal ailment	Cosmetic	Local brew	Others	IUCN Red List Status
A. leptosiphon	Back pain, Colic, Cough, Diabetes, Female infertility, Headache, Heart diseases, Hernia, Joint pain, Malaria, Blood pressure, Rashes, Tooth ache, Tooth cavity, Typhoid, Ulcers, Wounds.	Chicken (Newcastle disease), Cattle *East Coast Fever*			Lubricant for wooden tires, Pesticide (for vegetables)	CR
Aloe macrosiphon	Abscess/ boil, Blood clotting after getting a wound, Cleaning the stomach, Cough, Flue, Fungus, Numbness (*Ganzi*), Heart diseases (control heart beats), Bilharzia, Malaria, Dysmenorrhea, Pimples, Stomachache, Typhoid, Worms, Wounds, Yellow fiver					NE
A. deserti	Ear ache, Fever, Kidney problems, Malaria, Rashes, Spleen problems, Stomach ache, Wounds	Cattle pneumonia, Cattle *East Coast Fever*		Alcohol	Uvula (*Kilimi*), Tiredness	VU
A. rabaiensis	Bile, Ear ache, Fever, Kidney, Liver problems, Malaria, Rashes, Spleen, Stomach ache, Ulcers, Pneumonia, Wounds	Cattle *East Coast Fever*, Cattle pneumonia		Alcohol	Tiredness	LC
A. parvidens	Malaria, Wounds				Nairobi fly bite, Tiredness	LC
A. ballyi	Cough, Fever, Malaria, Stomach ache, Ulcers, Wounds			Alcohol	Tiredness, To remove jiggers from the body	EN

Scientific name	Human ailment	Animal ailment	Cosmetic	Local brew	Others	IUCN Red List Status
A. secundiflora	Bile, Cancer, Colic, Cough, Diabetes, Ear ache, Eye ache, Fever, Gas in the stomach, Gonorrhea (STDS), Hernia, Kidney, Leg pain, Malaria, Pneumonia, Rashes, Spleen, STD's, Stomach ache, Toothache, Wounds	Chicken (Newcastle disease), Cattle (gas in the stomach), Cattle *East Coast Fever*		Alcohol	To remove oil in meat soup, Tiredness, To remove Uvula (*Kilimi*)	LC
A. volkensii spp. volkensii	Diabetes, Malaria, Stomach ache, Wounds	East Coast Fever, Chicken (Newcastle disease)				LC
A. volkensii var multicalius	Cough, Diabetes, Eye ache, Halitosis, Hernia, Malaria, Pneumonia, Spleen, STD's, Stomach ache, Tooth ache, Wounds	Cattle constipation (*Kitasura*- Chaga), Cattle *East Coast Fever*, Chicken (Newcastle disease)		Alcohol	To prevent bad eyes and allows bananas to flourish (*local beleives*), Tiredness, To clean the bowel, To remove oil in meat soup	LC

Scientific name	Human ailment	Animal ailment	Cosmetic	Local brew	Others	IUCN Red List Status
A. bicomitum	Amoeba, *Bengu* (Mpima in Fipa-yellow feces accompanied with blood and swollen/tumor in the stomach especially for children), Cough, Fever, Flue, Headache, STD's, Stomachache, Typhoid, Poison, Wounds	Chicken (Newcastle disease)				NE
A. christianii	Amoeba, Bengu, Cough, Ear ache, Ear pas, Fever, Hernia, Malaria, Ringworms, Stomach ache, Swellings, Swollen legs, Typhoid, Wounds	Chicken (Newcastle disease)			Sharp pain (*Kichomi*), Sprains, Drunkenness, Poison	NE
A. mzimbana	Stomach ache, Bengu, Fever, Hernia, Pungu/Ndondo- (worms coming from the anus), Typhoid, Wounds	Chicken (Newcastle disease)	Malassezia furfur (Utangotango wa ngozi)		Speed up labor and delivery	NE
A. chabaudii	Heart problems (too many heart beat that normal), Hernia, Stomach ache, Typhoid	Chicken (Newcastle disease)			Drunkenness	NE
A. duckerii	Bengu, Burns, Colic, Cough, Pungu, Ear ache, Ear pus, Fever, Headache, Hernia, Ovulation Disorder (O.D), Ringworms, Stomach ache, Typhoid, Ulcers, Wounds	Chicken (Newcastle disease)	Malassezia furfur (Utangotango wa ngozi)		Against snake bite, Poison, Sprains, Speed up labor and delivery	NE

Scientific name	Human ailment	Animal ailment	Cosmetic	Local brew	Others	IUCN Red List Status
Aloe (Unidentified-SA 45)	Hernia, Malaria, Stomach ache, Wounds	Chicken (Newcastle disease)				
Aloe (Unidentified-SA 53)	Malaria	Chicken (Newcastle disease)				

Appendix 4: All re-assessed *Aloe* species with year of collection, region, locality, and available specimens in international herbaria. The description of the Index Herbariorum: Meise Botanic Garden (BR), Royal Botanic Gardens (K), Missouri Botanical Garden (MO), Swedish Museum of Natural History (S), Desert Botanical Garden (DES), Naturalis (WAG) Aarhus University (AAU), Senckenberg Gesellschaft für Naturforschung: Senckenberg Forschungsinstitut und Naturmuseum (FR). Siri Abihudi (SA)

Scientific name	Collector	Year of collection	Region	Locality	Specimens in Western herbaria (# of specimens)
A. ballyi	S.Abihudi	2018	Kilimanjaro	Lembeni ward in Mwanga district, Mamba ward in Same district	K (3), MO (2)
A. bicomitum	S.Abihudi	2018	Rukwa	Kasanga, Muzi, Namanyele	MO (1), S (1)
A. boscawenii	S.Abihudi	2018	Tanga	Manza, Moa, and Boma	
A. chabaudii	S.Abihudi	2018	Katavi	Tanganyika district	MO (2)
A. christianii	S.Abihudi	2018	Katavi and Rukwa	Tanganyika, Mchakamchaka, Msanzi, Muze, Kasanga, Namanyele, and Mpanda	MO (1)
A. confusa	S.Abihudi	2018	Kilimanjaro	Lake Chala	K (6)
A. deserti	S.Abihudi	2018	Kilimanjaro	Lembeni wards in Mwanga district and Kisiwani ward in Same district	K (7), MO (3)
A. dorotheae	S.Abihudi	2018	Tanga	Bangu, Kideleko, Kilimamzinga,	DES (2), MO (2)

Scientific name	Collector	Year of collection	Region	Locality	Specimens in Western herbaria (# of specimens)
				Kwamkono, Msasa, Kwamagome, Hoza,	
A. duckeri	S.Abihudi A.Chawala	2018 2019	Katavi, Rukwa, Iringa, and Njombe	Msukumilo, Matai, Msanzi, Laela, Namanyele, Mpanda, Njombe, Mufindi, Ugala River,	MO (3)
A. fibrosa	S.Abihudi	2018	Kilimanjaro	Simba farm in Engarenairobi ward	K (3)
A. flexilifolia	S.Abihudi	2018	Tanga	Soni, Kisiwani village	MO (4)
A. lateritia	S.Abihudi	2018	Mara, Kilimanjaro, and Tanga	Kisangula, Pongwe, Handei, Magamba, Kwematungutu, Migulu, Shukilai, Migambo, Kifulilo, Kifungiro, Masange, Gale, Kisangula, Handei, Bangu, Kwamkono, Kwamagome, Golani, Handeni, Mbamba, Hoza,Kwafivi, Mshizii, Kwamsononi, Soni, Lushoto, Bomole hill, Amani, Rombo, Dodoma, and Mbulu.	K (51), MO (6)
A. leptosiphon	S.Abihudi	2018	Tanga	Soni, Kishewa, Magila, Kwamsononi, Mgombelwa, and Mlalo	MO (4)

Scientific name	**Collector**	**Year of collection**	**Region**	**Locality**	**Specimens in Western herbaria (# of specimens)**
A. macrosiphon	S.Abihudi	2018	Mara	Serengeti NP	K (1), MO (7)
A. massawana	S.Abihudi	2018	Tanga, Dar-es-Salaam, Pemba	Pongwe, Moa, Boma, Manza, Mwera, Dar-es-Salaam near sea cliff hotel, Masange village, and Pemba	MO (7)
A. mzimbana	S.Abihudi	2018	Rukwa	Mtai, Msanzi, Laela, Namanyele, Ugala River, and Tunduma	FR (9)
A. myriacantha	S.Abihudi	2018	Arusha and Rukwa	Lake Duluti, Sopa Chala	K (14), MO (2)
A. nuttii	S.Abihudi	2018	Rukwa	Muzi	MO (2), WAG (2)
A. parvidens	S.Abihudi	2018	Kilimanjaro	Kiverenge, Lembeni	
A. rabaiensis	S.Abihudi	2018	Kilimanjaro	Jipe and Lembeni	MO (1)
A. secundiflora	S.Abihudi	2017, 2018	Mara, Kilimanjaro, Arusha, Manyara, Dodoma	Serengeti NP, Kisangula, Engarenairobi, Siha, Ngarenanyuki, Jipe, Kisiwani, Stelingi, Mwembe, Sanya juu, Chemka, close to lake Chala, Himo, Kiverenge, Lembeni, Holili and Mwika, Arumeru, Mbulu, Kongwa	AAU (1), BR (1), DES (3), MO (2), WAG (2)

Scientific name	Collector	Year of collection	Region	Locality	Specimens in Western herbaria (# of specimens)
A. volkensii.	S.Abihudi	2017, 2018	Mara, Manyara, Kilimanjaro, Arusha, and Tanga	Karatu, Mwembe, Mbaga, Bangu, Mwika, Handei, Pongwe, Serengeti NP, Kisangula, Kisiwani, Same, Himo, Siha, Tongoni, Lushoto, Marangu, Lembeni, Kiverenge, Arusha, Arumeru, Golani, Kifulilo, Mtindili, Mamba, Ngoni village, Nkwisha,	AAU (1), K (8), MO (9)

Appendix 5: Consent form: English version

STUDY TITLE: SYSTEMATIC REVIEW AND TOXICITY STUDIES OF TANZANIAN ALOE.

My name is Siri Abihudi, a 1st year student doing PhD Degree at The Nelson Mandela African Institute of Science and Technology, Arusha).
I am conducting a study with the above title as part of my study program.

Aims of the study

This study aims to '**Systematic Review and do the toxicity studies of Tanzanian Aloe**. This will help the public to know the potential and effect that can result from using *Aloe* species.

Participation in this study:

During this study, you are requested to respond to some questions about *Aloe* species. This interview is designed to take less than half an hour. Feel free to expand on the topic or talk about related ideas. Also, if there are any questions you would rather not answer or that you do not feel comfortable answering, please say so and we will skip the question and move on to the next question, whichever you prefer or decline the interview altogether.

Risks:

We do not anticipate any risks involved in participating in this study

Benefits:

By participating in this study, awareness about the use and the effects resulting from uses will be revealed. The uses will be tested in the laboratory and the Aloe with potential uses will be communicated to the community for cultivation and for development.

Confidentiality:

All the personal information will be kept confidential. Only we and the research team will have access to the material. Results of our study will be published in an Open Access Journal and presented on scientific conferences. After completion of this project, all materials will be destroyed or stored at a secure location.

Cost:

You will not be required to make any payments to participate in this study and no payment will be made to you.

For further information, questions or queries, you can contact:

1. The student researcher,

   Siri Abihudi

   NM-AIST.

   Arusha

   Cell no: +255 712 958 211

   Email: sabihudi@gmail.com

Participant's Agreement

I,____, have read/been told of the contents of this form and have understood its subject. I agree to participate in this study.

Signature of participant ________________________

Signature of Researcher ________________________

Date____

Appendix 6. Questionnaire to the respondents

Date ………...

a. Biodata

Village		Name	
Age		Religion	
Gender		Tribe	
Education		Main occupation	

Collection and uses of *Aloe* species

1. How long have you been here? …………………………………………………
2. Do you know *Aloe*? ....
3. What is the local name? ……………………………………………………
4. Can you differentiate them? Yes / No
5. How? ……………………………………………………………………………
6. Do you know their usefulness? Yes / No
7. How frequently do you harvest *Aloe*

   a. Once per week

b. Once per months

c. Once per six month

d. Once per year

8. Are the *Aloe* harvested from the wild or cultivated?.........................(If cultivated go to qn 10)

9. How far is from here to where you can find the *Aloe*?

a. Less than 1Km

b. 1km

c. 2km

d. More than 2km

10. How many *Aloe* can u collect for use in the wild…………………………..

11. What can u say about the availability of *Aloe* spp in the wild

a. Widely available

b. Intermediately available

c. Rare/ difficult to get

12. Is livestocks affecting the survival of *Aloe*? Yes/No………

13. Is there diseases threatening *Aloe* species?.........Which one?.................... (fungus?)

14. How do you insure the availability of *Aloe* around you?…………….

a. Good Harvesting techniques………………………………..

b. *Aloe* in Home garden………………………………………….

c.

15. Do you have aloe in your home garden/farm?.................

16. How many individuals are in your garden/farm?.......................

17. Is there a trade of *Aloe* in your area?…………………..

18. Is it in high demand or low?................................

19. How much is the *Aloe* sold per leave?

    a. 100-500

    b. 600-1000

    c. 1000-1500

    d. Above 1500

20. What are the threat factors to *Aloe* spp……………………………………………………..
21. When is flowering period for *Aloe* ……………………………………………………..
22. Please list the *Aloe* species and other information needed in the Table 1.

Tabe 1: list of *Aloe* species, management uses and their conservation

***Aloe* species**	**Management status** C=Cultivated W=Weed Wi=Wild	**Part used:** *Fr= Fruits* *S=Seeds* *Fl=Flowers* *L=Leaves* *SB=Stem* *RT = whole root* *WP=whole plant*	**Preparation** *PG-Peal and get the gel* *CS-Cut and Soak* *B-Boil* *Other*	**Uses and side effect** D-Drink A-Apply Others	**Status** *A- Abundant* *T-Threatened* *R-Rare* *E-Extinct*	**Disease treated**	**Management status** C=Cultivated W=Weed Wi=Wild	**Harvest method** *Up-Uprooting Aloe for the roots* *Cf-Cut a leaf plant* *Ct-Cut two leaves or more per plants* *Taking the sterm*	**Harvest pattern** 1. Harvest sparingly to conserve species; 2. do not care whether plant dies or not

23. Which of the above diseases have you treated in the last one year?

………………………………………………………………………………………………………

………………………………………………………………………………………………………

24. What were the outcomes of the treatment? ………

Thanks for your participation

Appendix 6: Species distribution maps for the 22 *Aloe* species studied in Tanzania. Layer data from the Tanzania National Bureau of Statistics, created with ArcGIS v.10.1 (https://www.arcgis.com, © Esri and its licensors, all rights reserved)

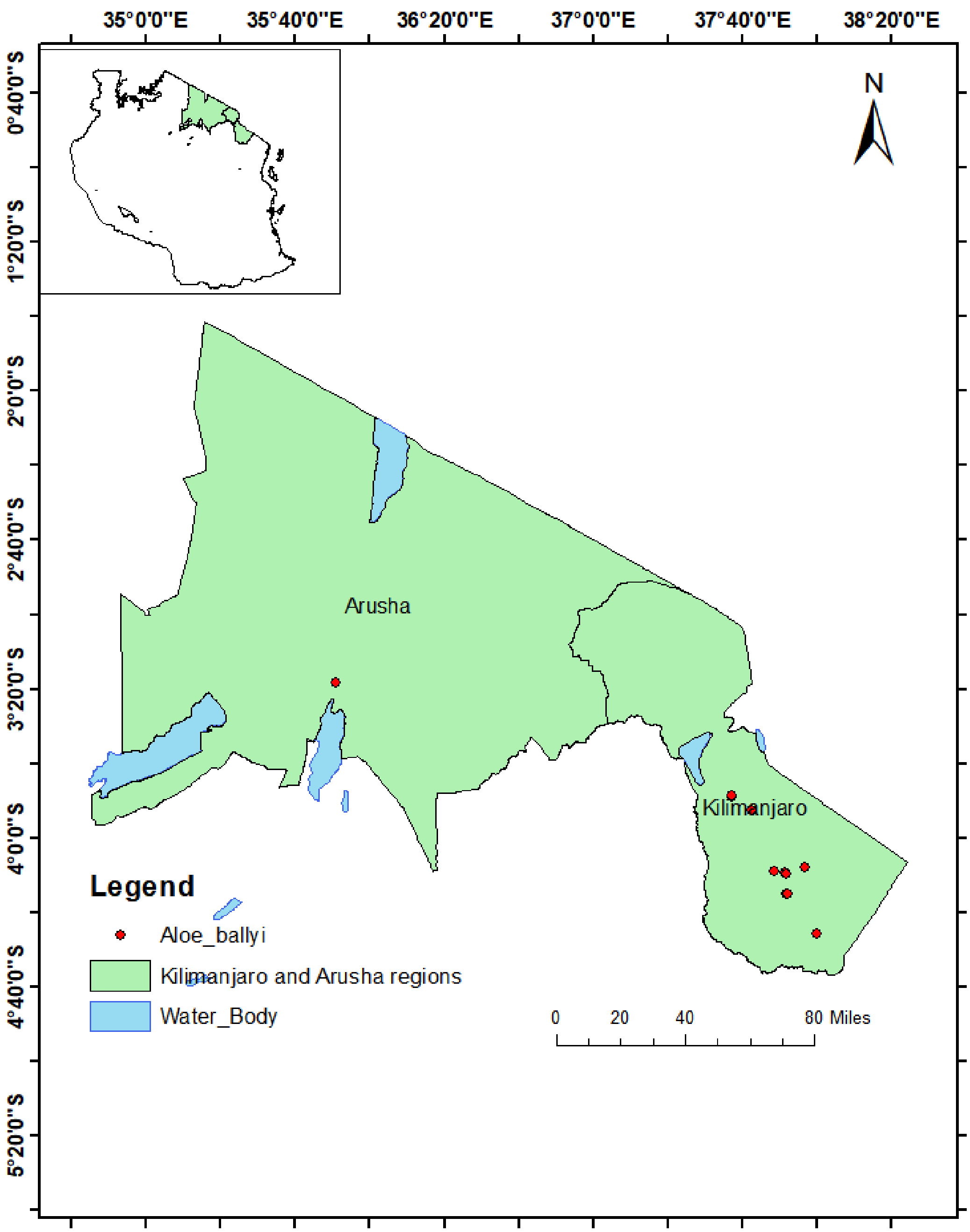

Figure 17: Species locality map for *Aloe ballyi* in Tanzania

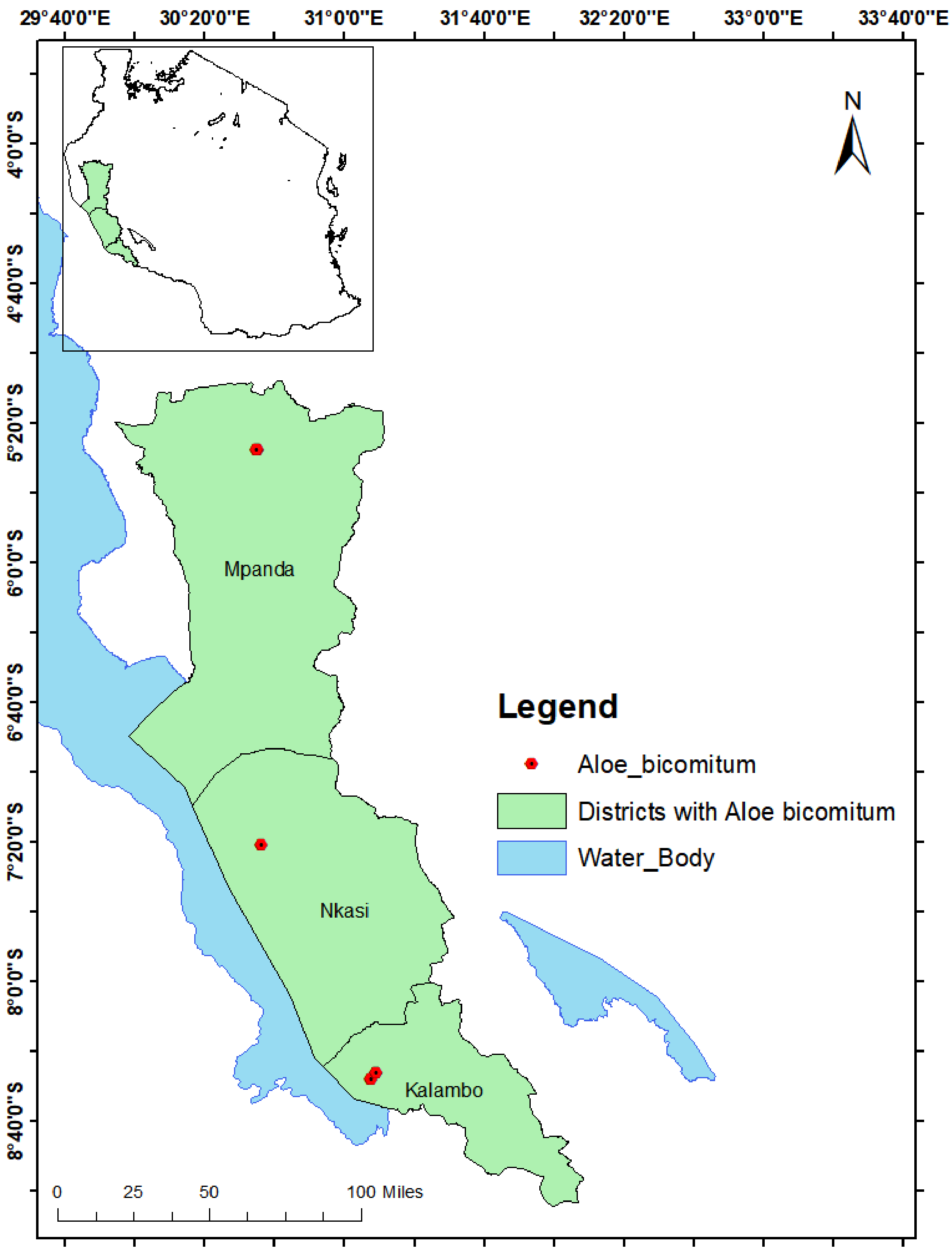

Figure 18: Species locality map for *Aloe bicomitum* in Tanzania

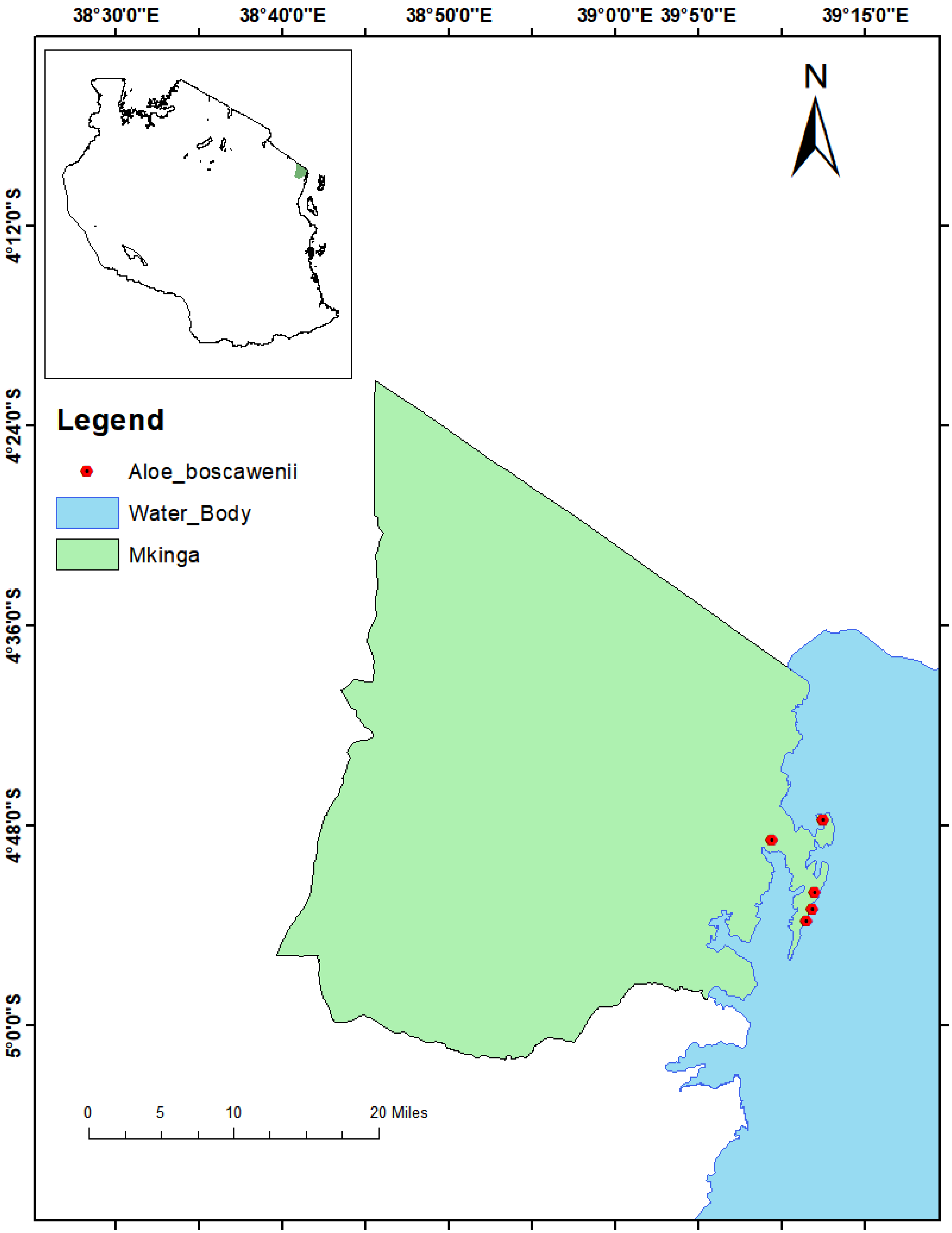

Figure 19: Species locality map for *Aloe boscawenii* in Tanzania

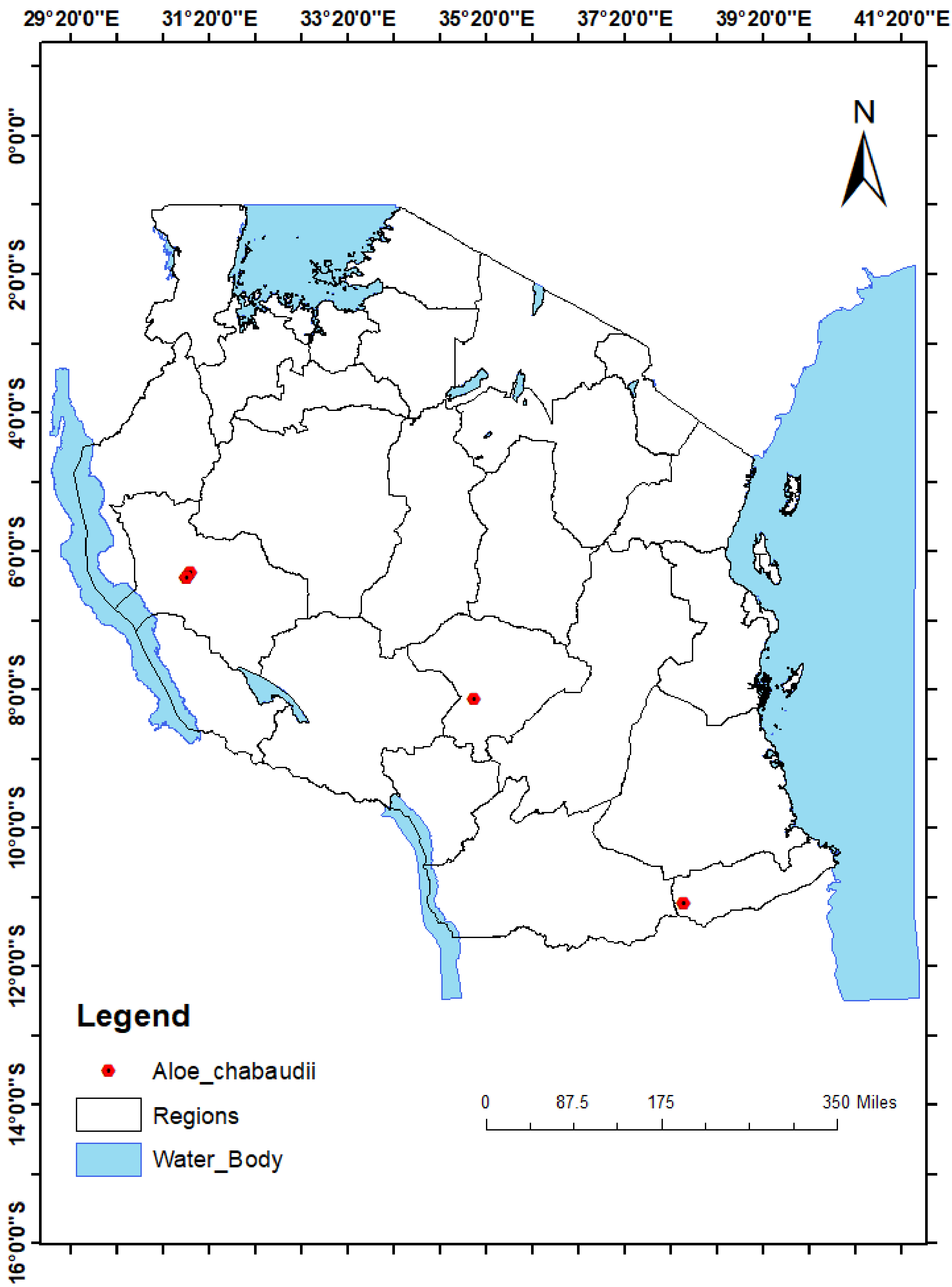

Figure 20: Species locality map for *Aloe chabaudii* in Tanzania

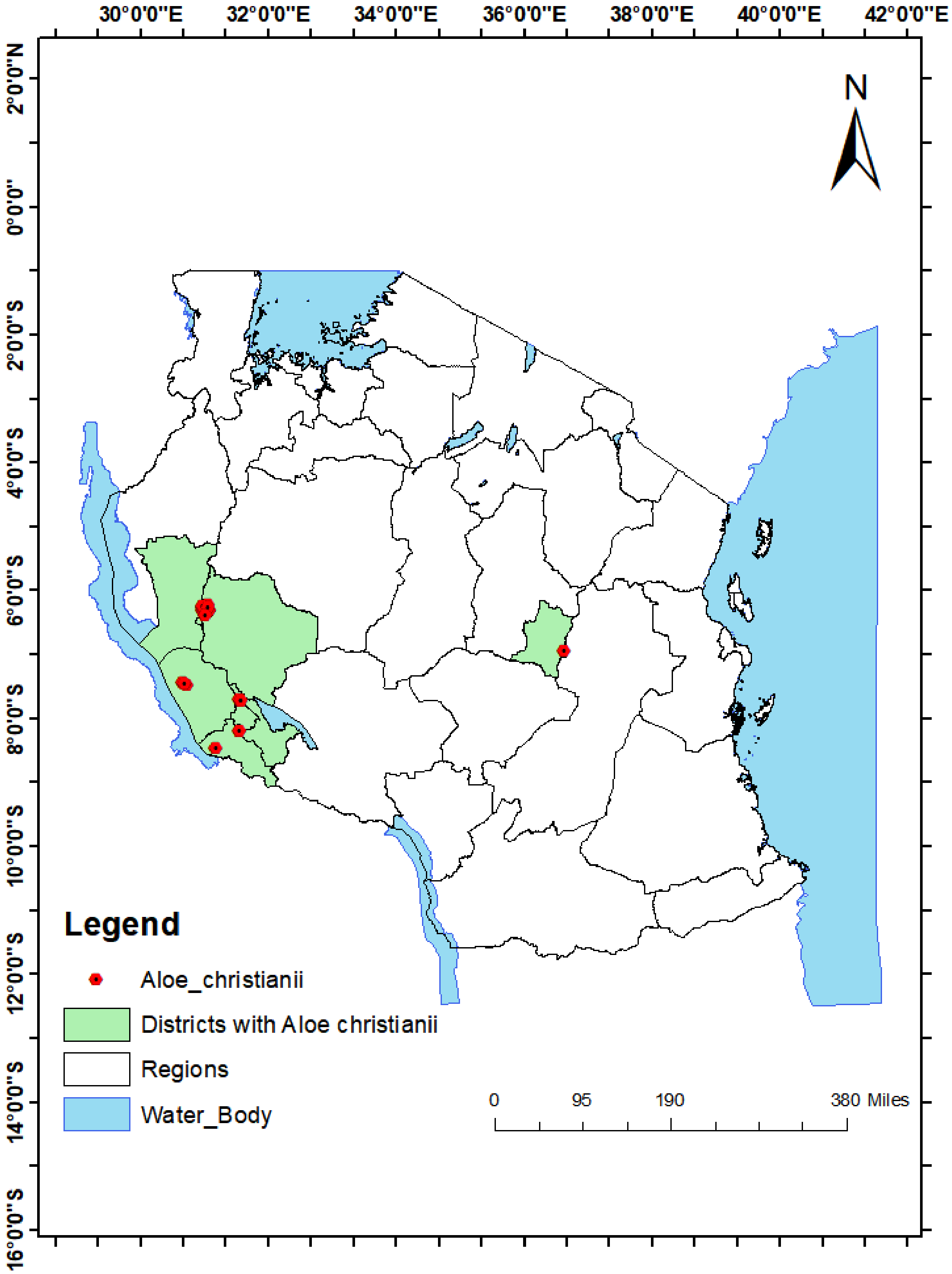

Figure 21: Species locality map for *Aloe christianii* in Tanzania

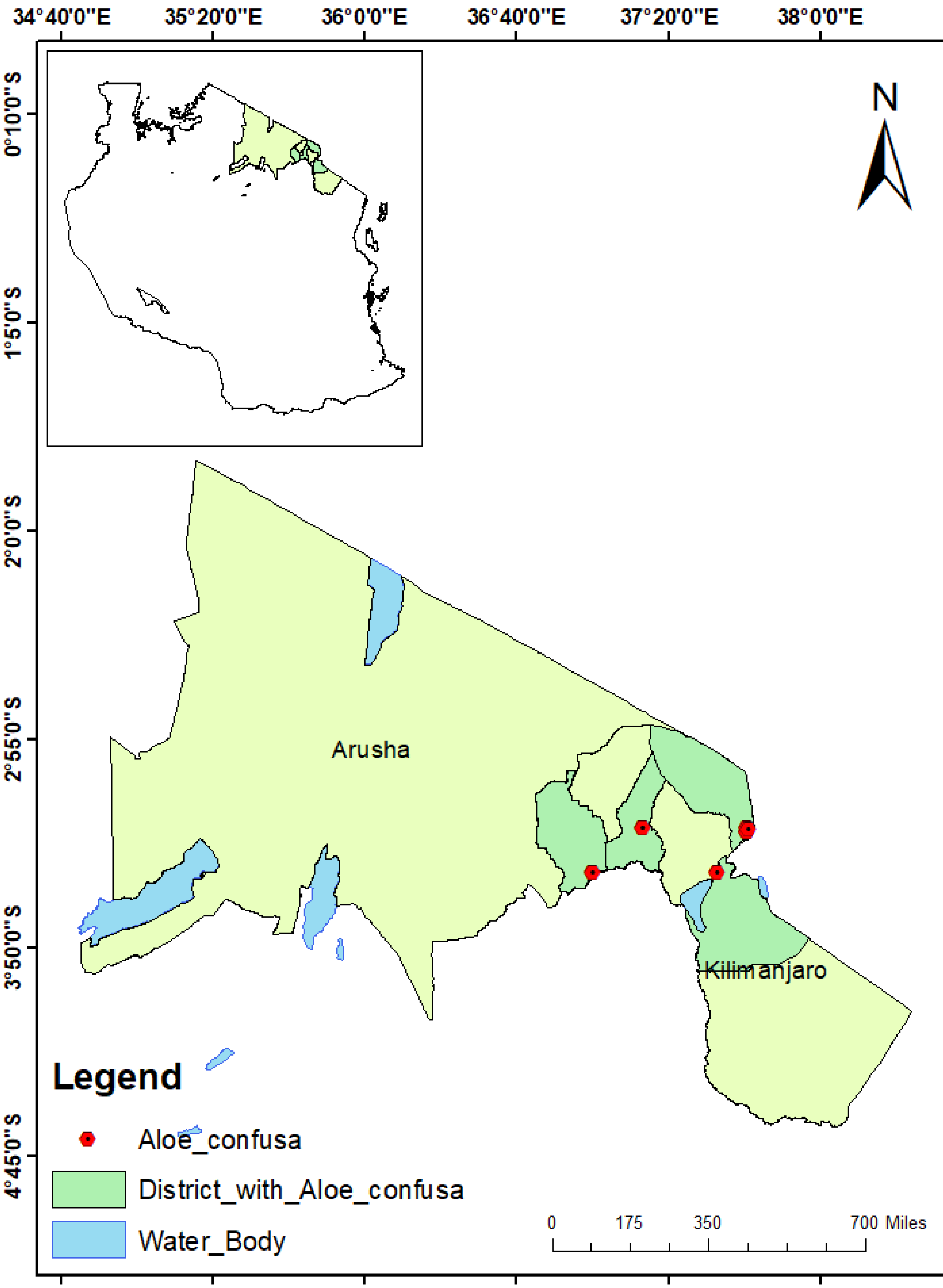

Figure 22: Species locality map for *Aloe confusa* in Tanzania

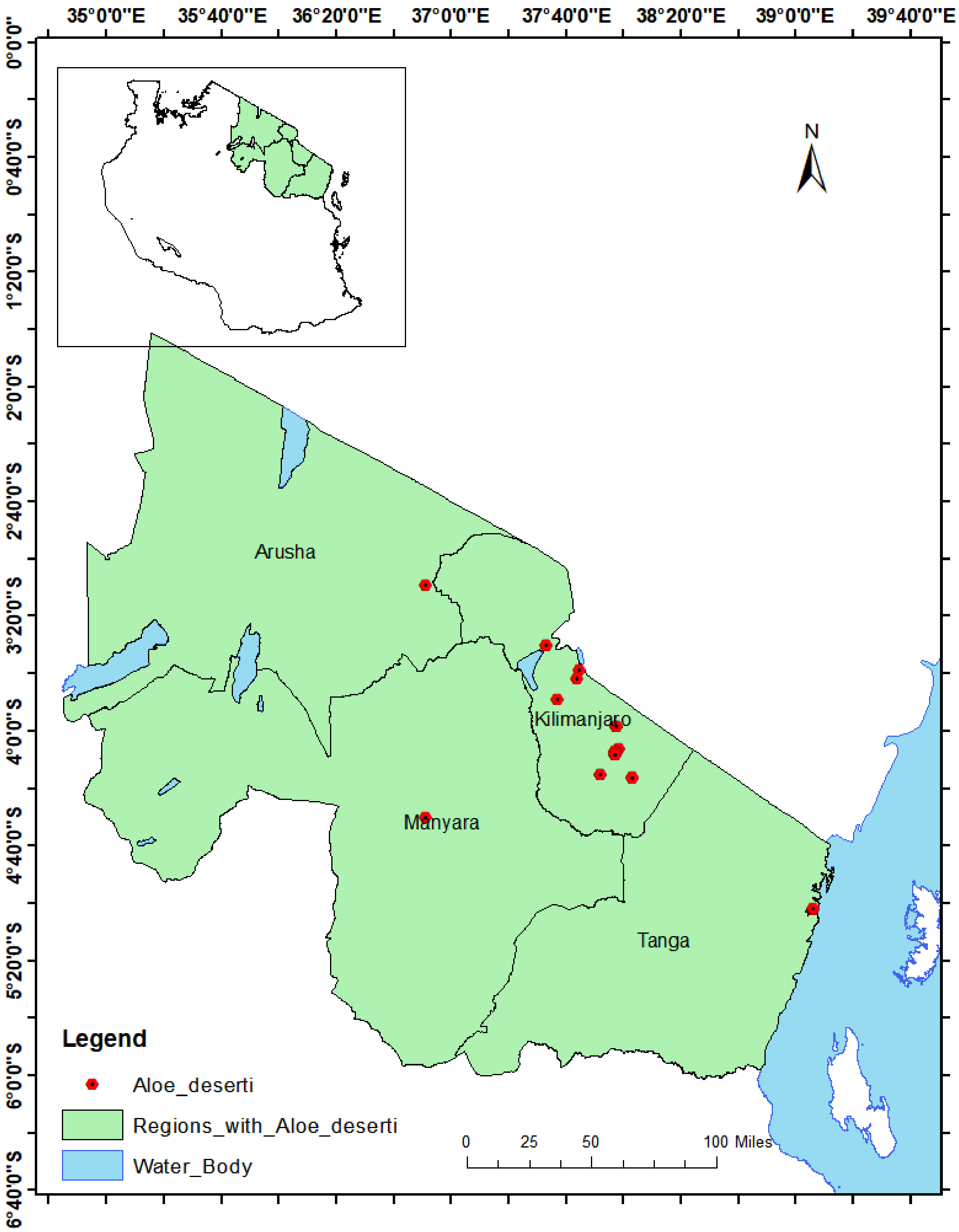

Figure 23: Species locality map for *Aloe deserti* in Tanzania

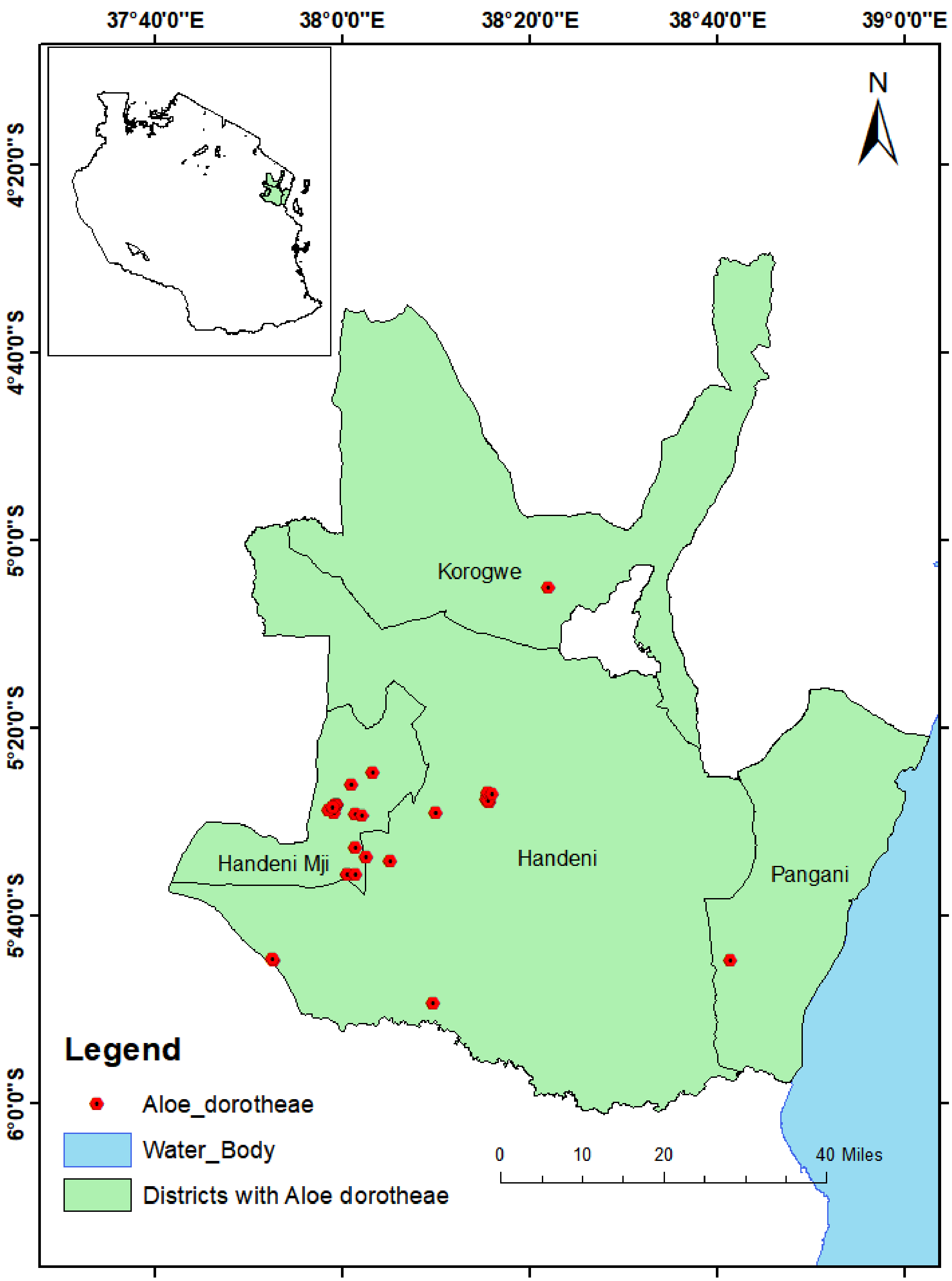

Figure 24: Species locality map for *Aloe dorotheae* in Tanzania

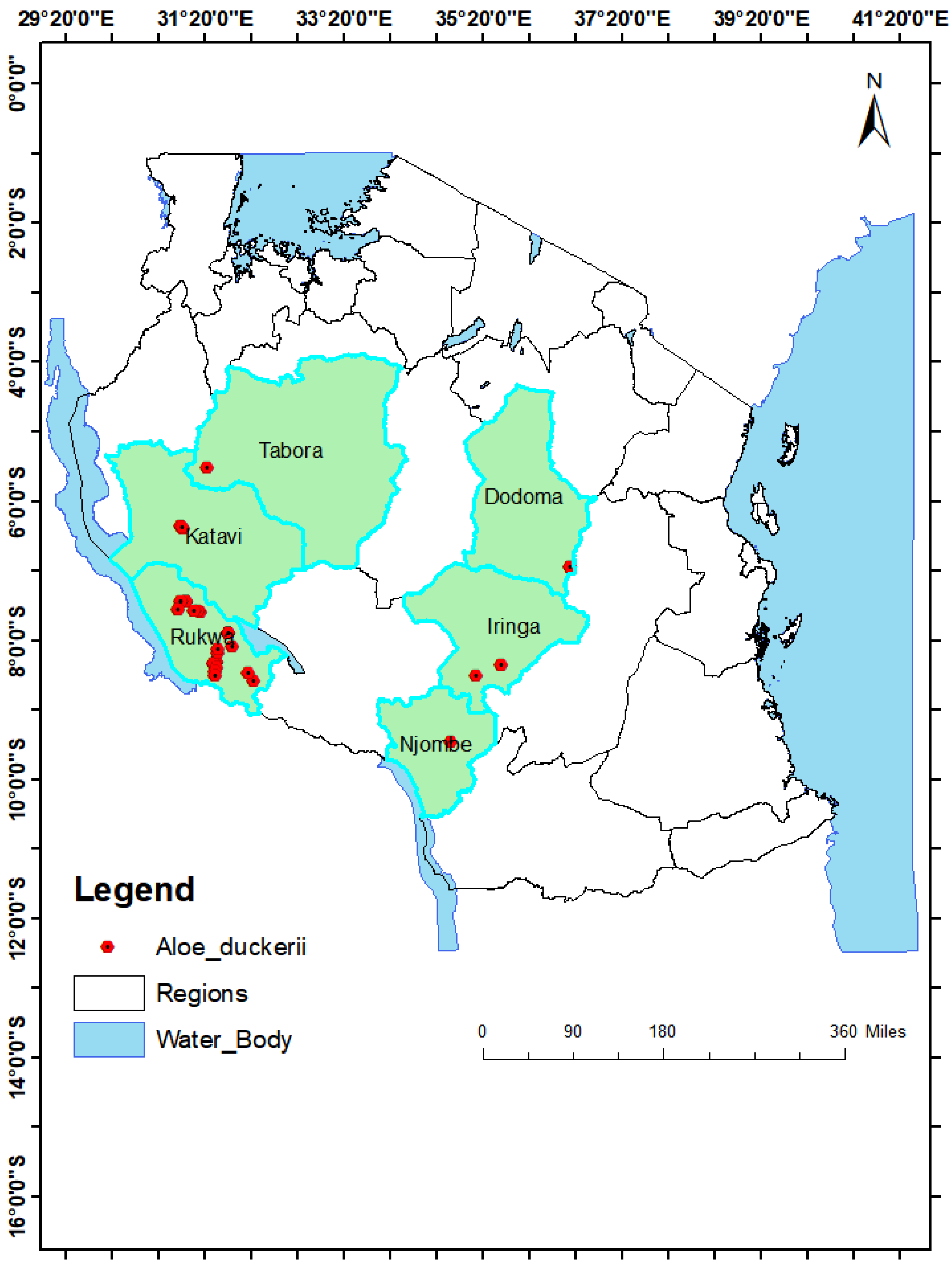

Figure 25: Species locality map for *Aloe duckerii* in Tanzania

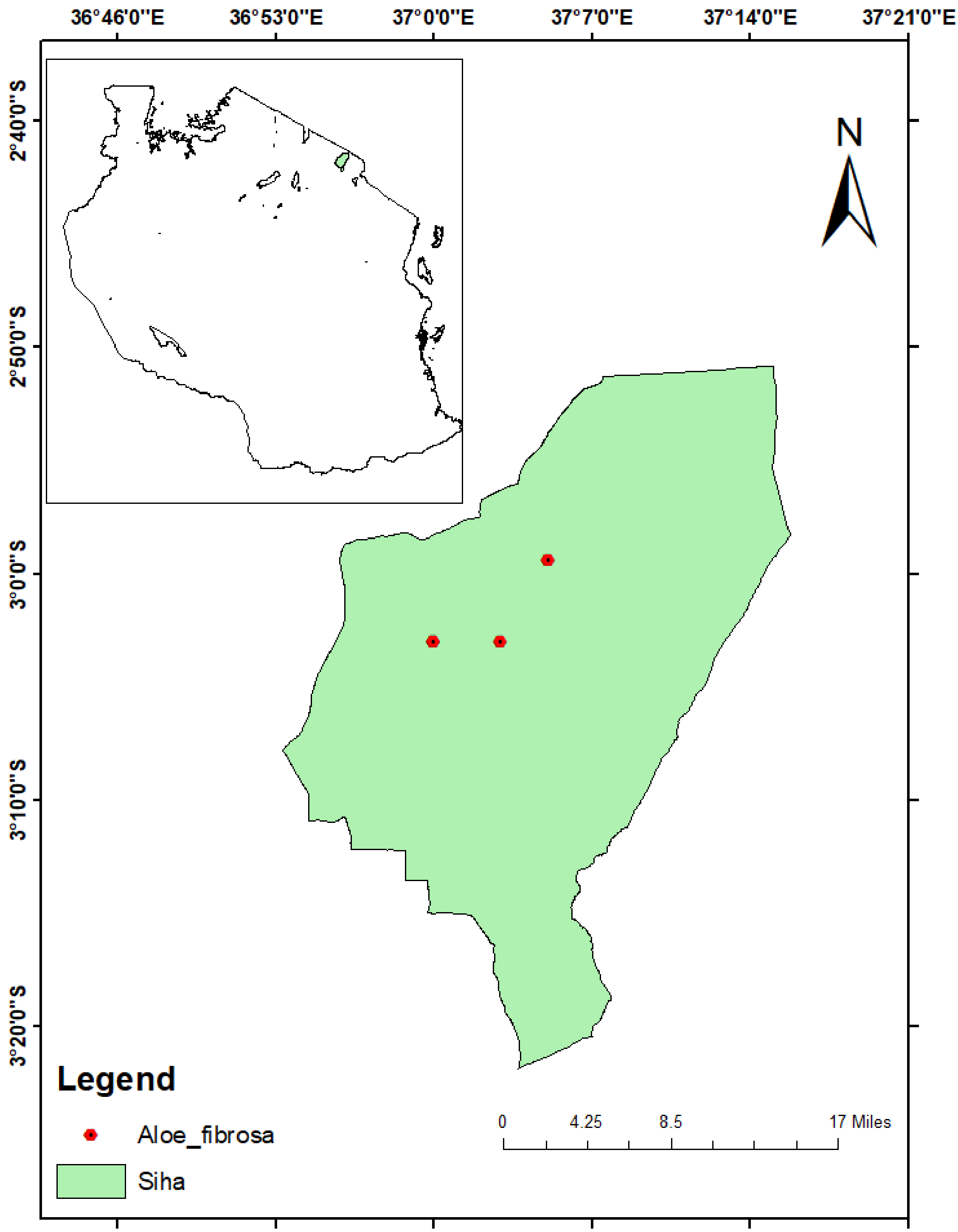

Figure 26: Species locality map for *Aloe fibrosa* in Tanzania

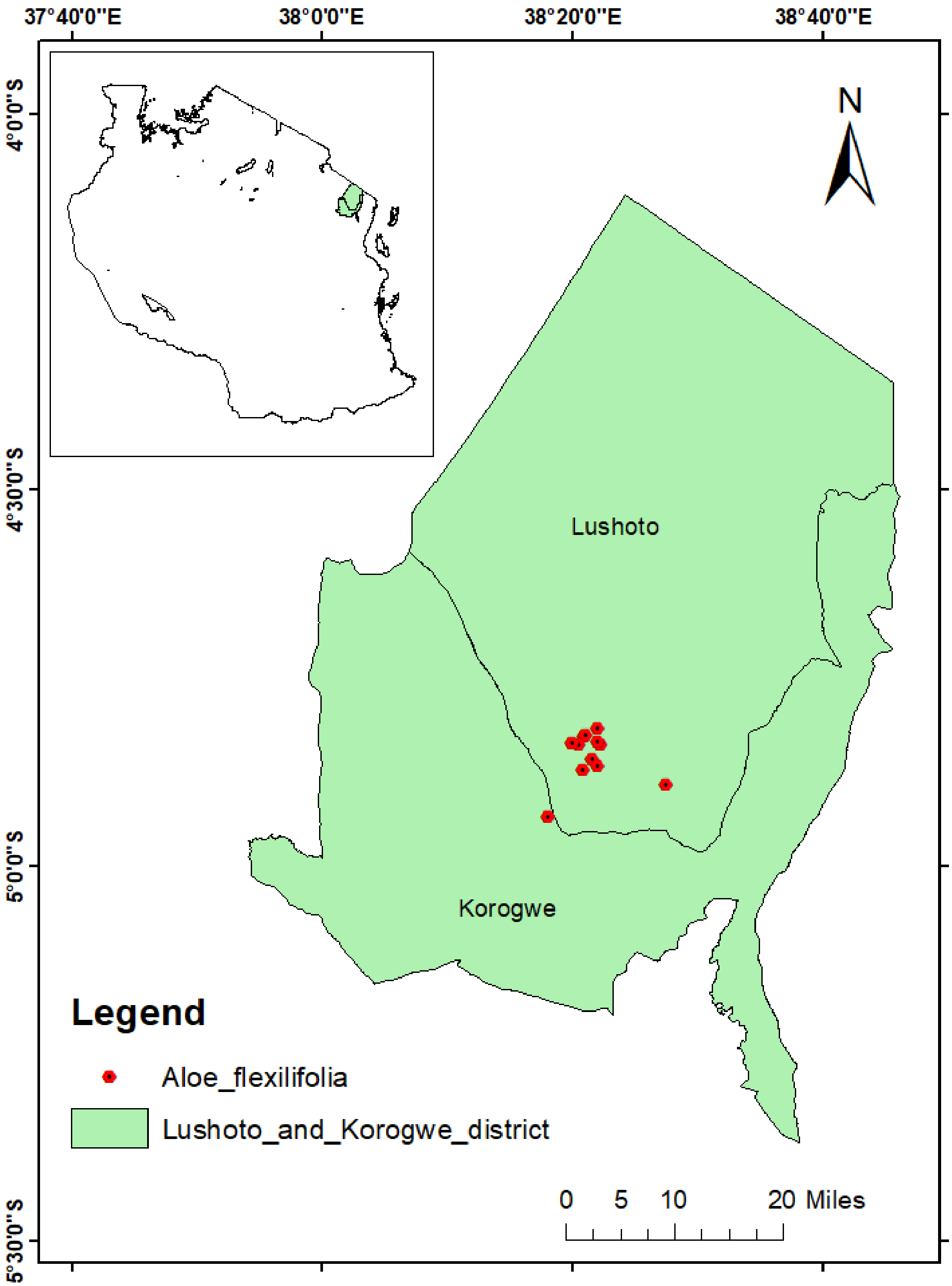

Figure 27: Species locality map for *Aloe flexilifolia* in Tanzania

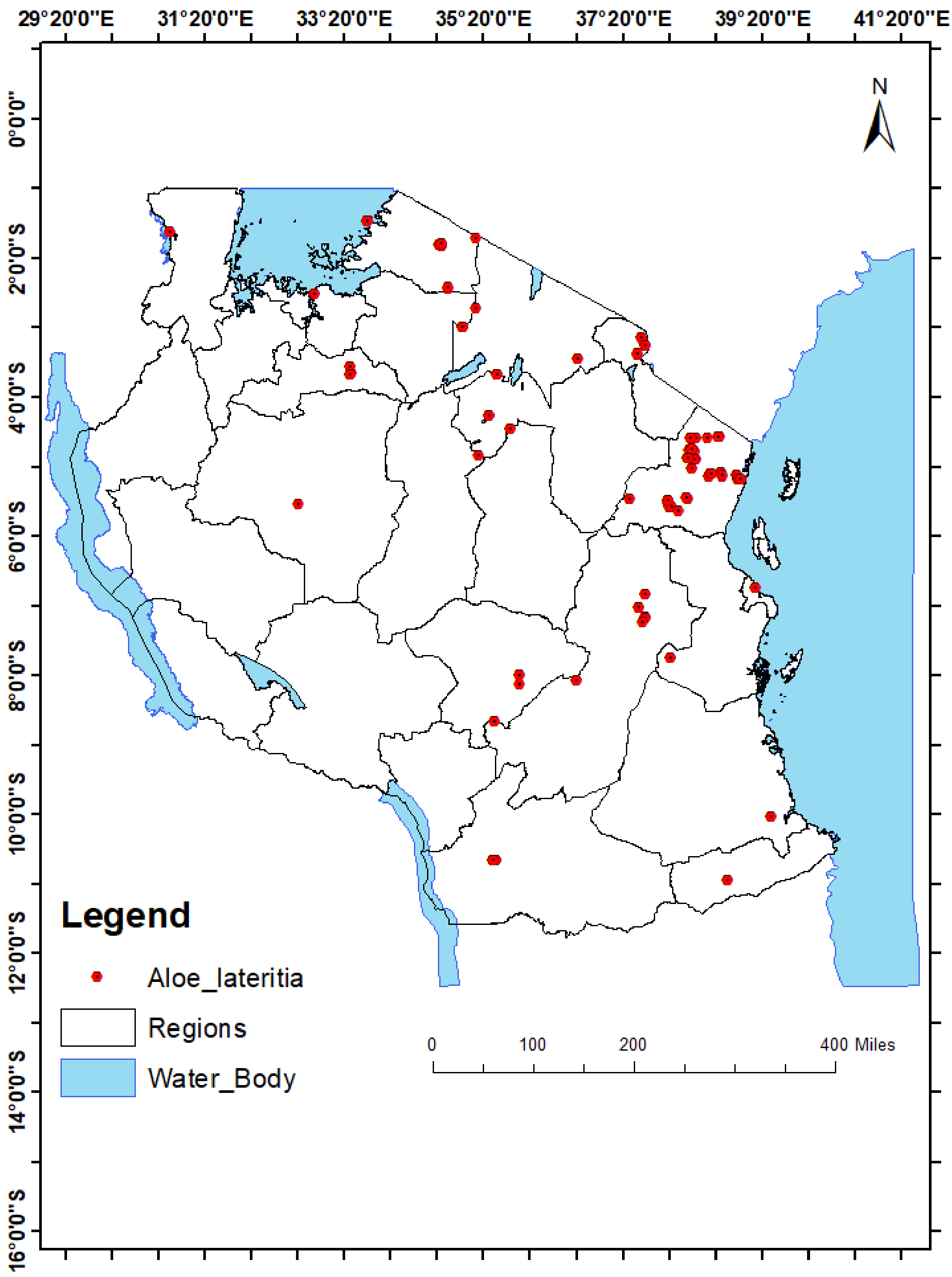

Figure 28: Species locality map for *Aloe lateritia* in Tanzania

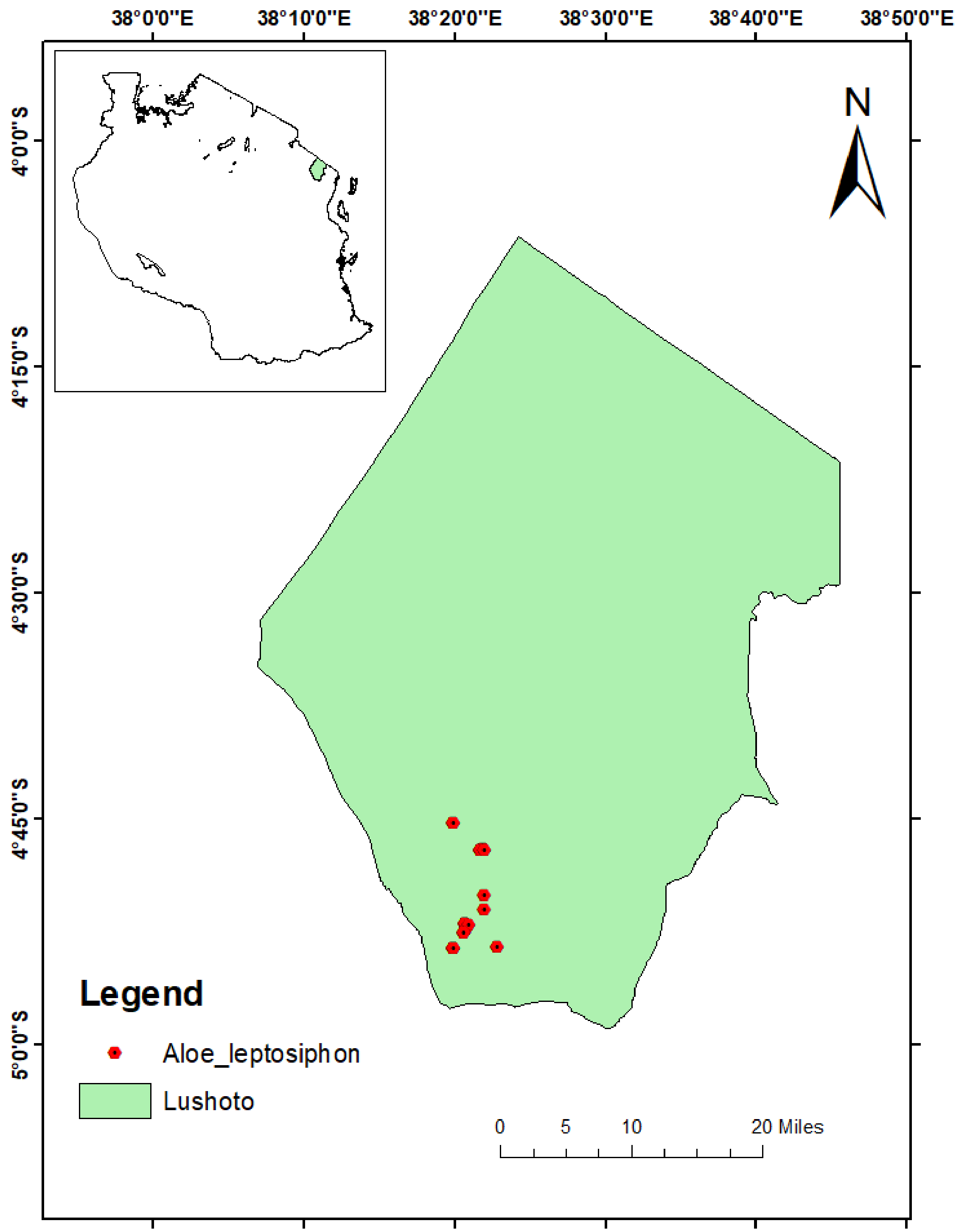

Figure 29: Species locality map for *Aloe leptosiphon* in Tanzania

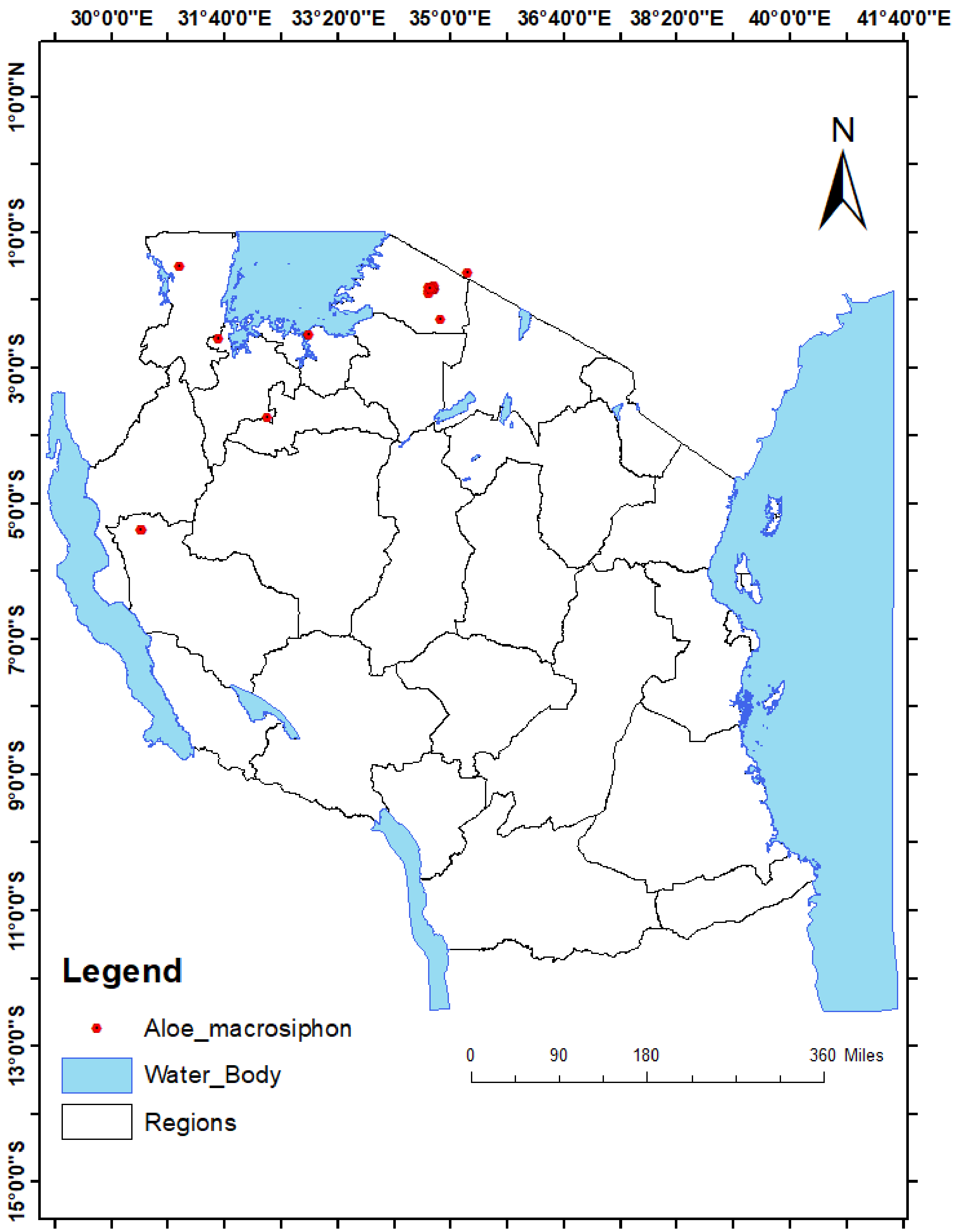

Figure 30: Species locality map for *Aloe macrosiphon* in Tanzania

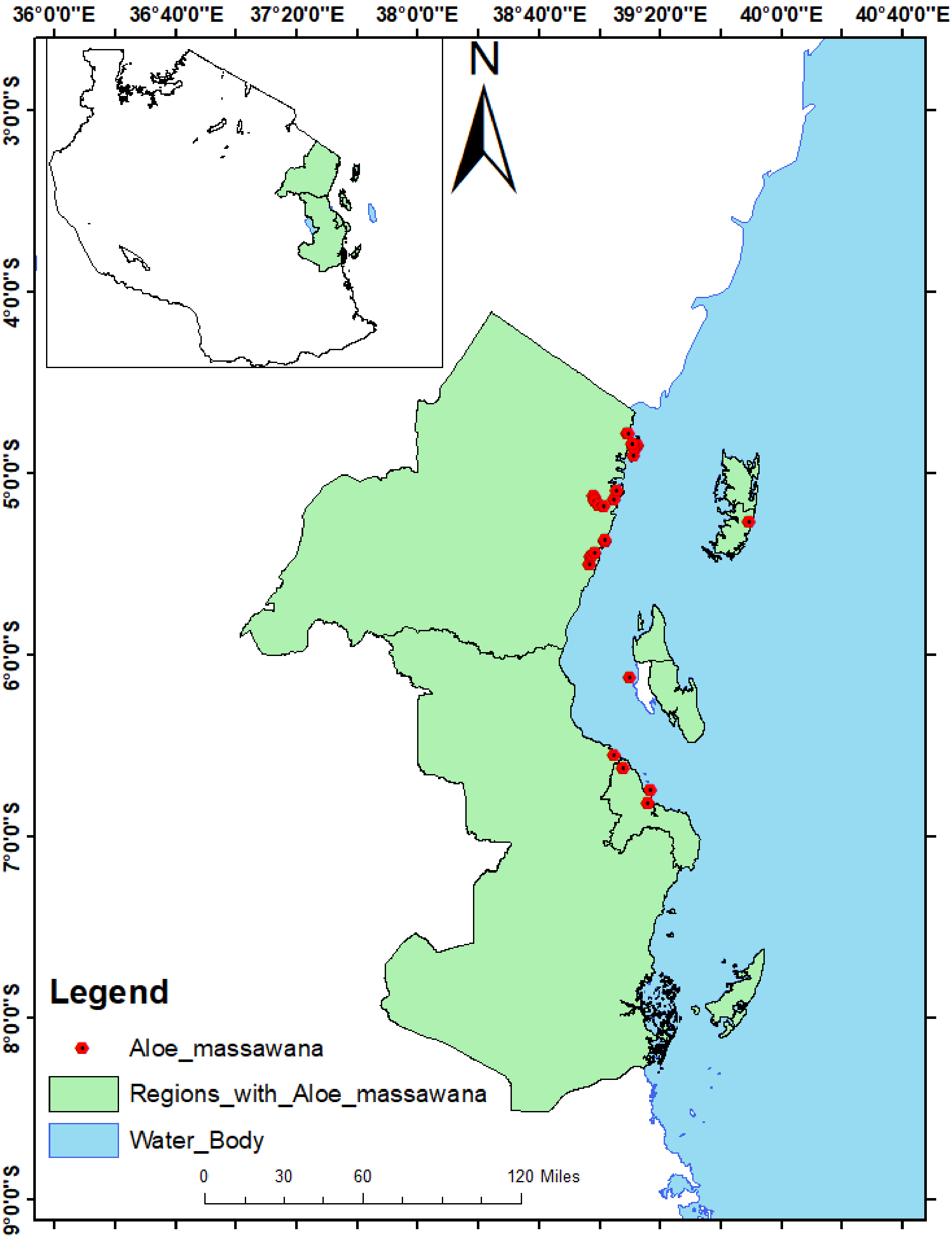

Figure 31: Species locality map for *Aloe massawana* in Tanzania

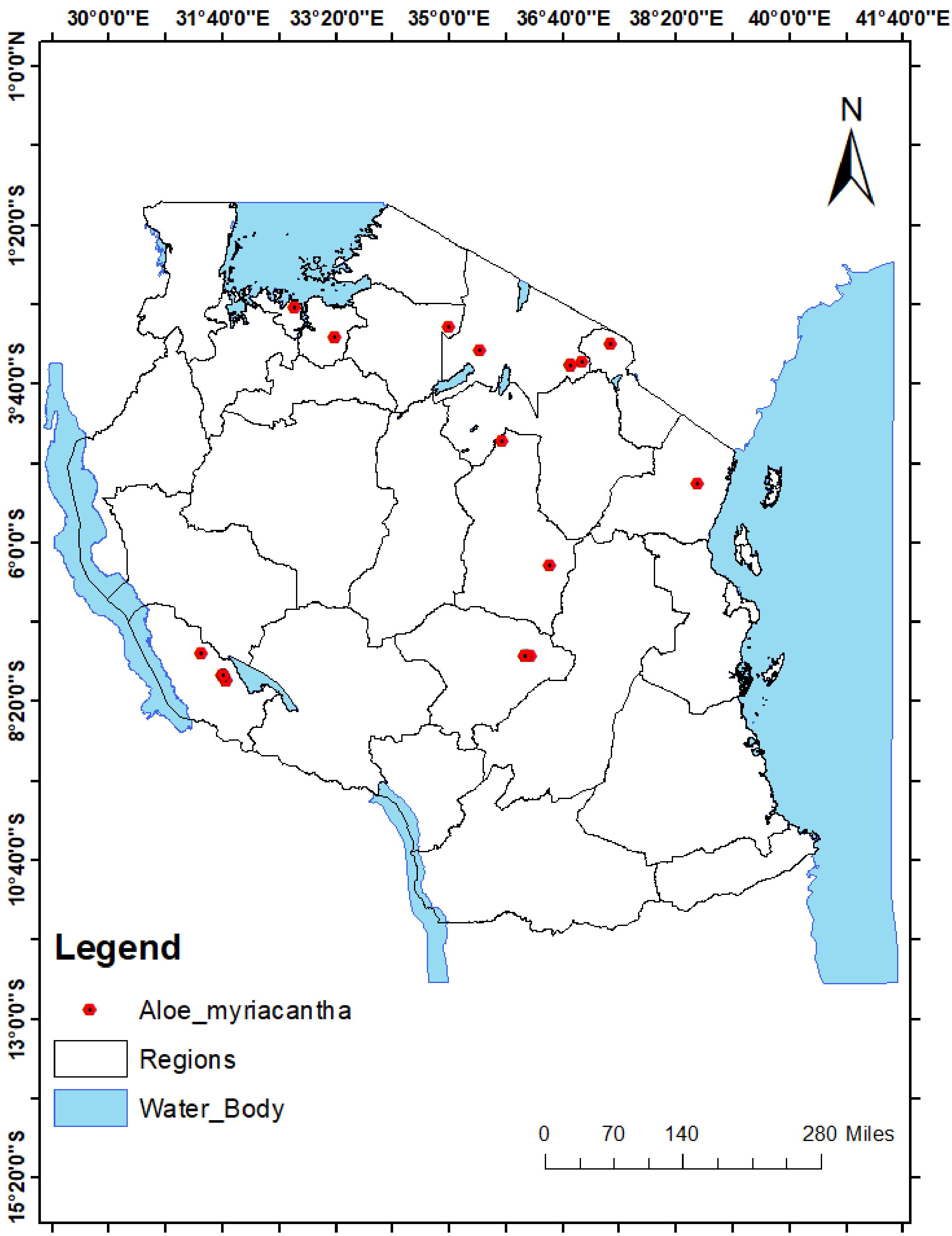

Figure 32: Species locality map for *Aloe myriacantha* in Tanzania

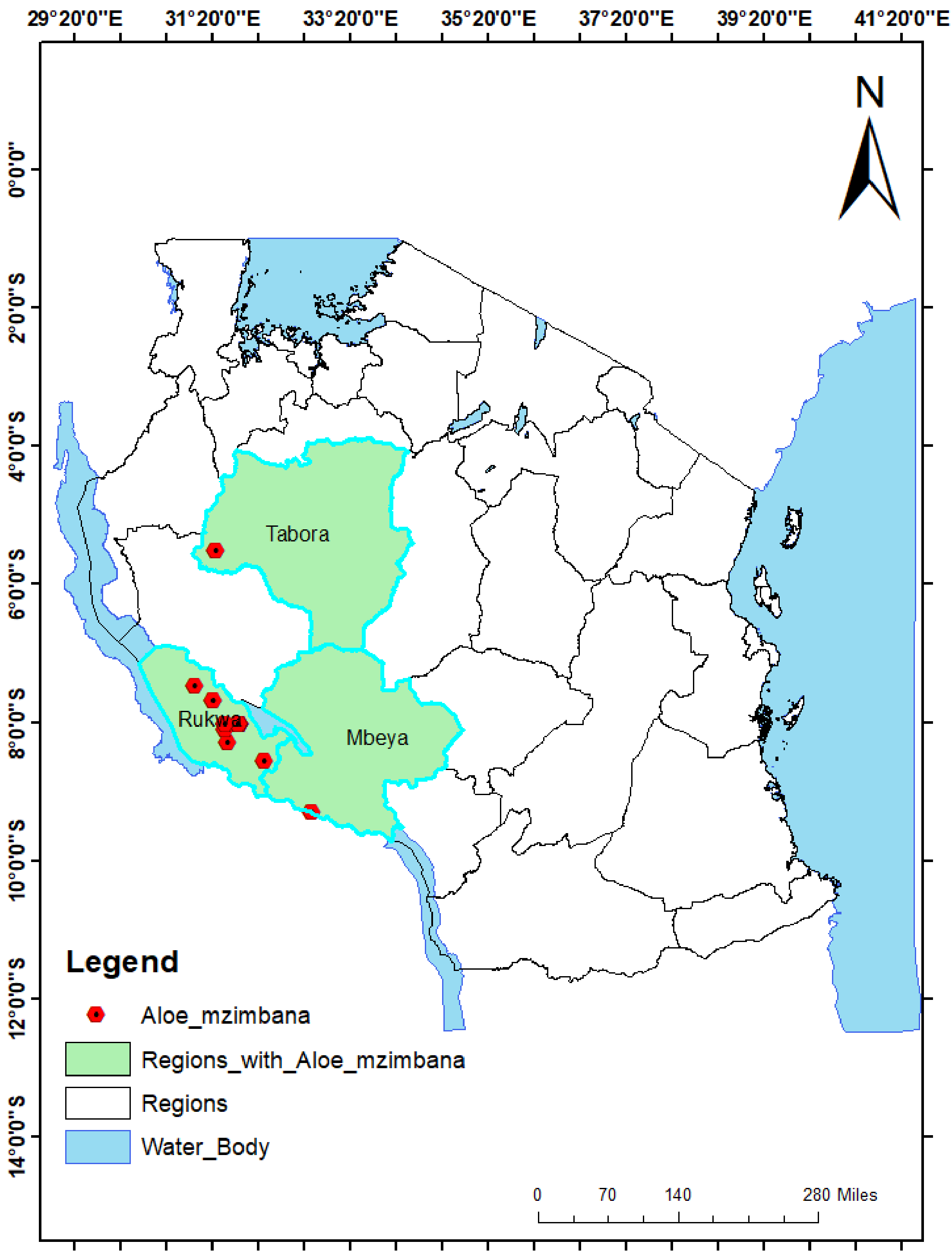

Figure 33: Species locality map for *Aloe mzimbana* in Tanzania

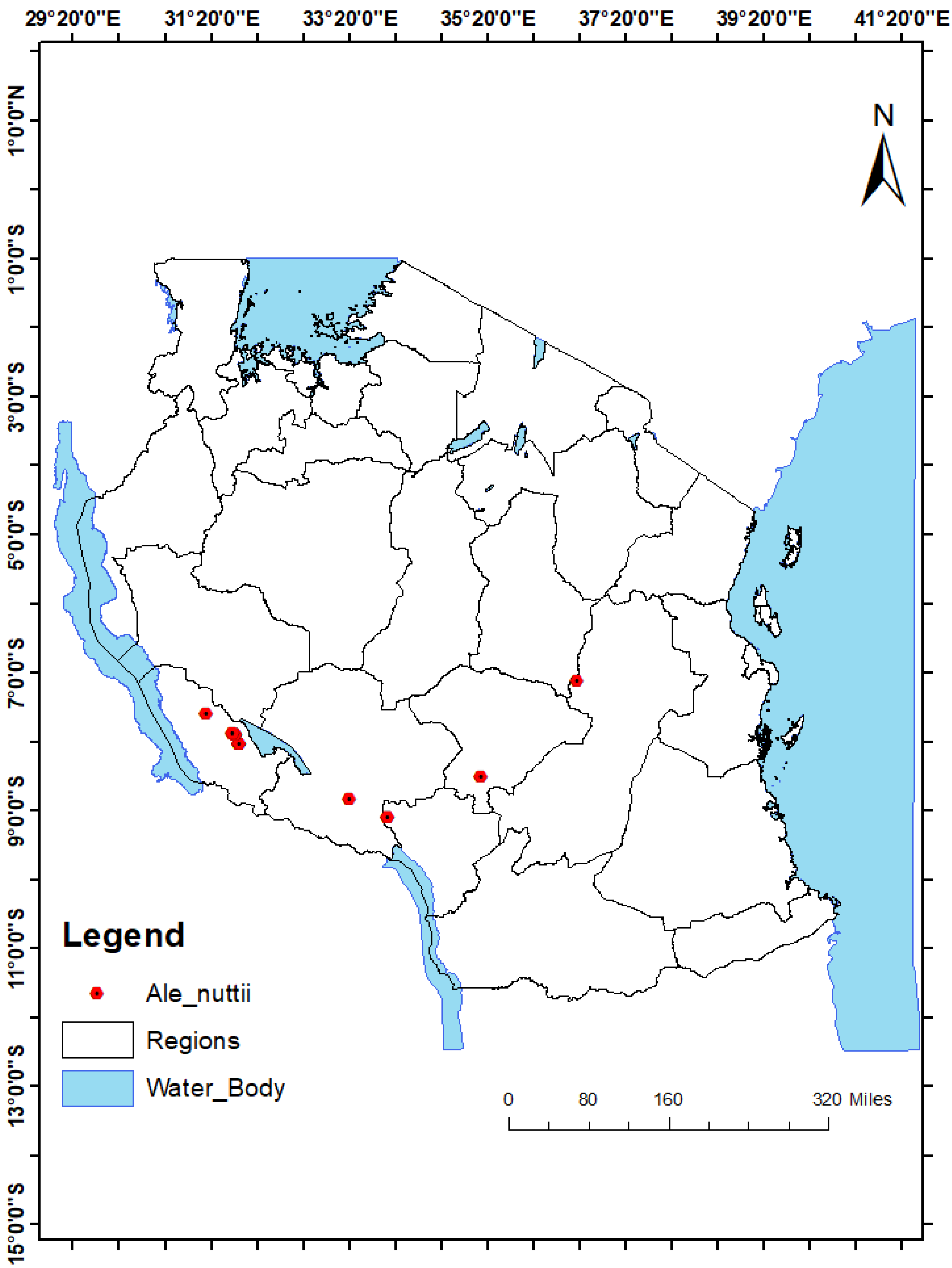

Figure 34: Species locality map for *Aloe nuttii* in Tanzania

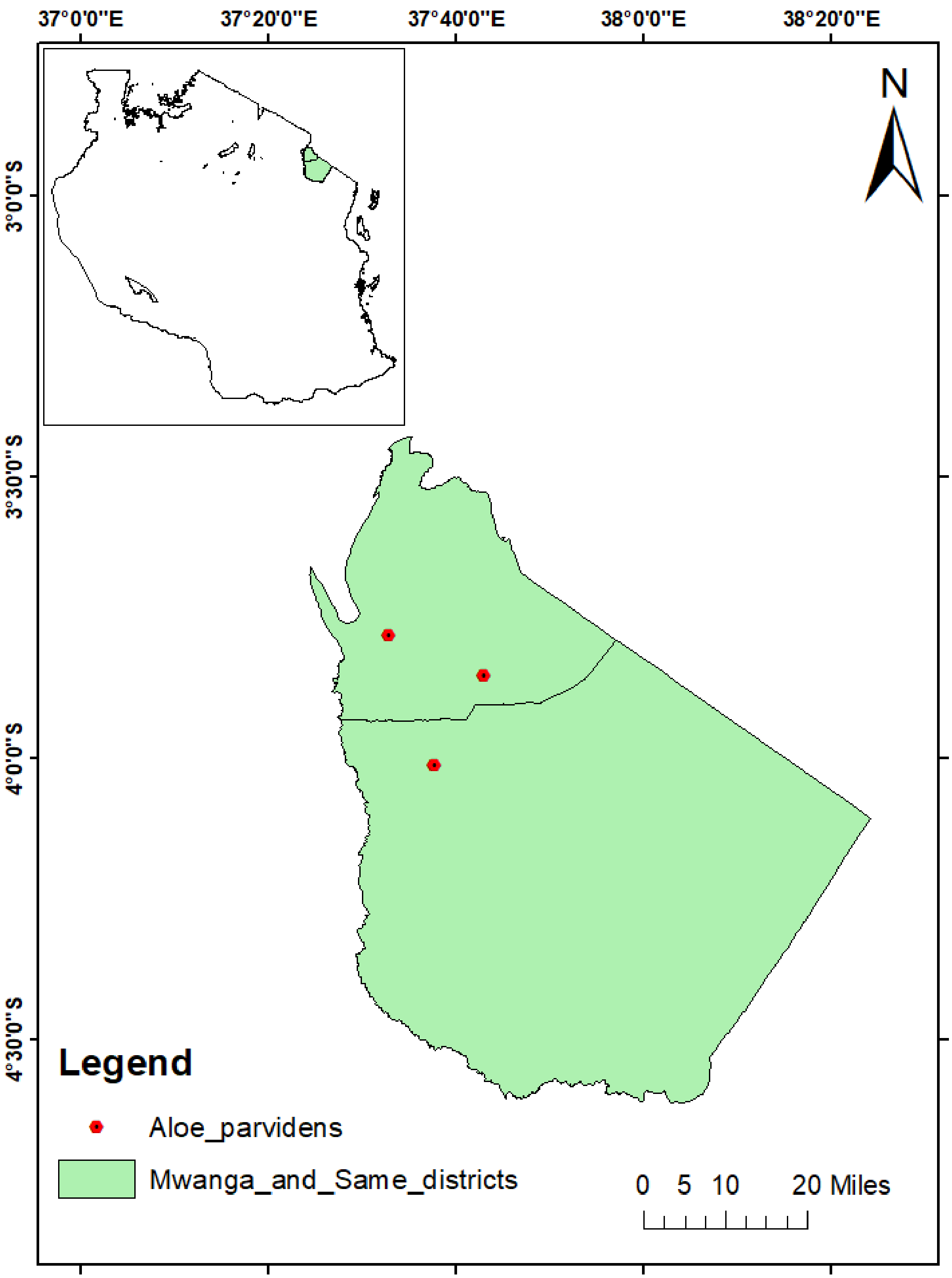

Figure 35: Species locality map for *Aloe parvidens* in Tanzania

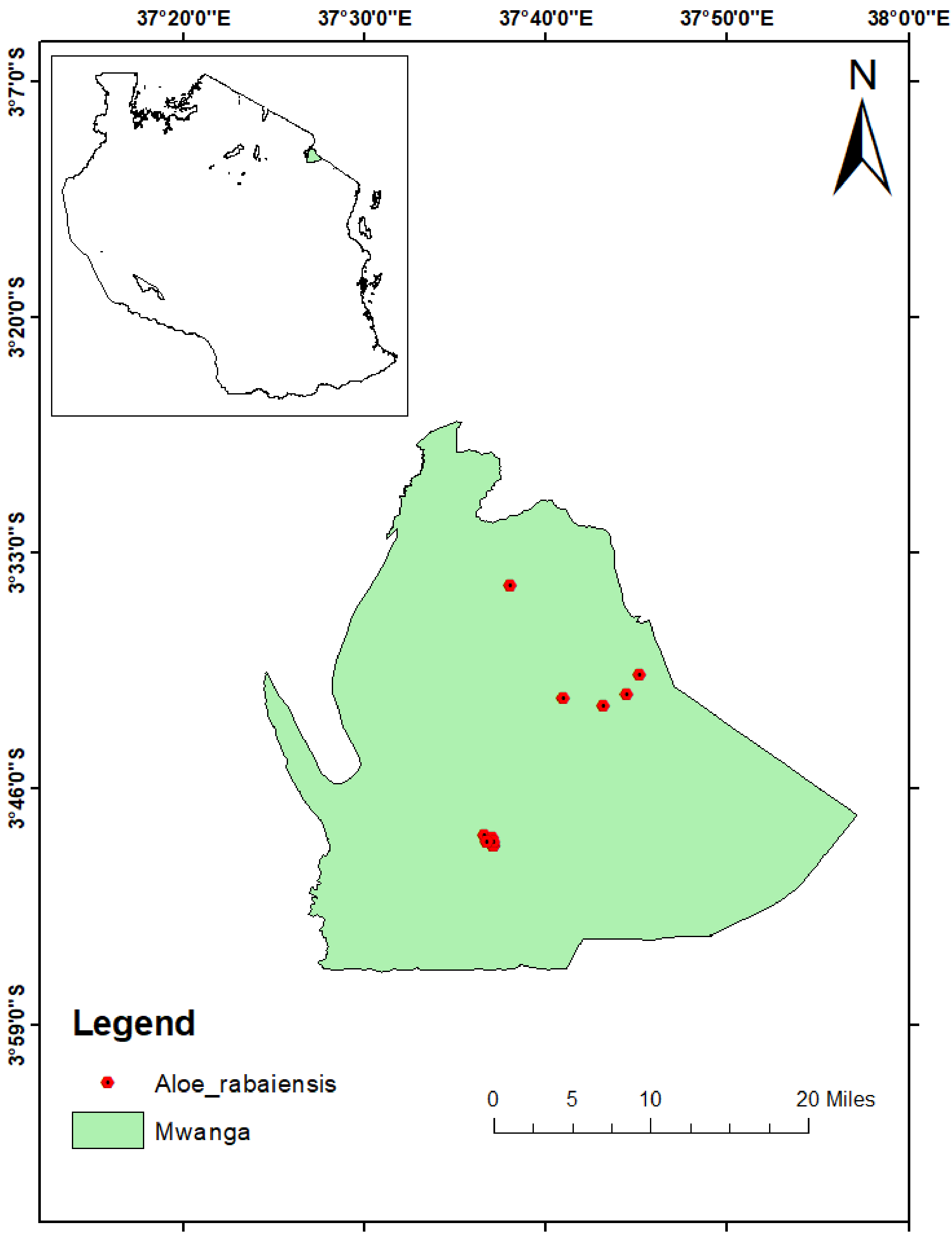

Figure 36: Species locality map for *Aloe rabaiensis* in Tanzania

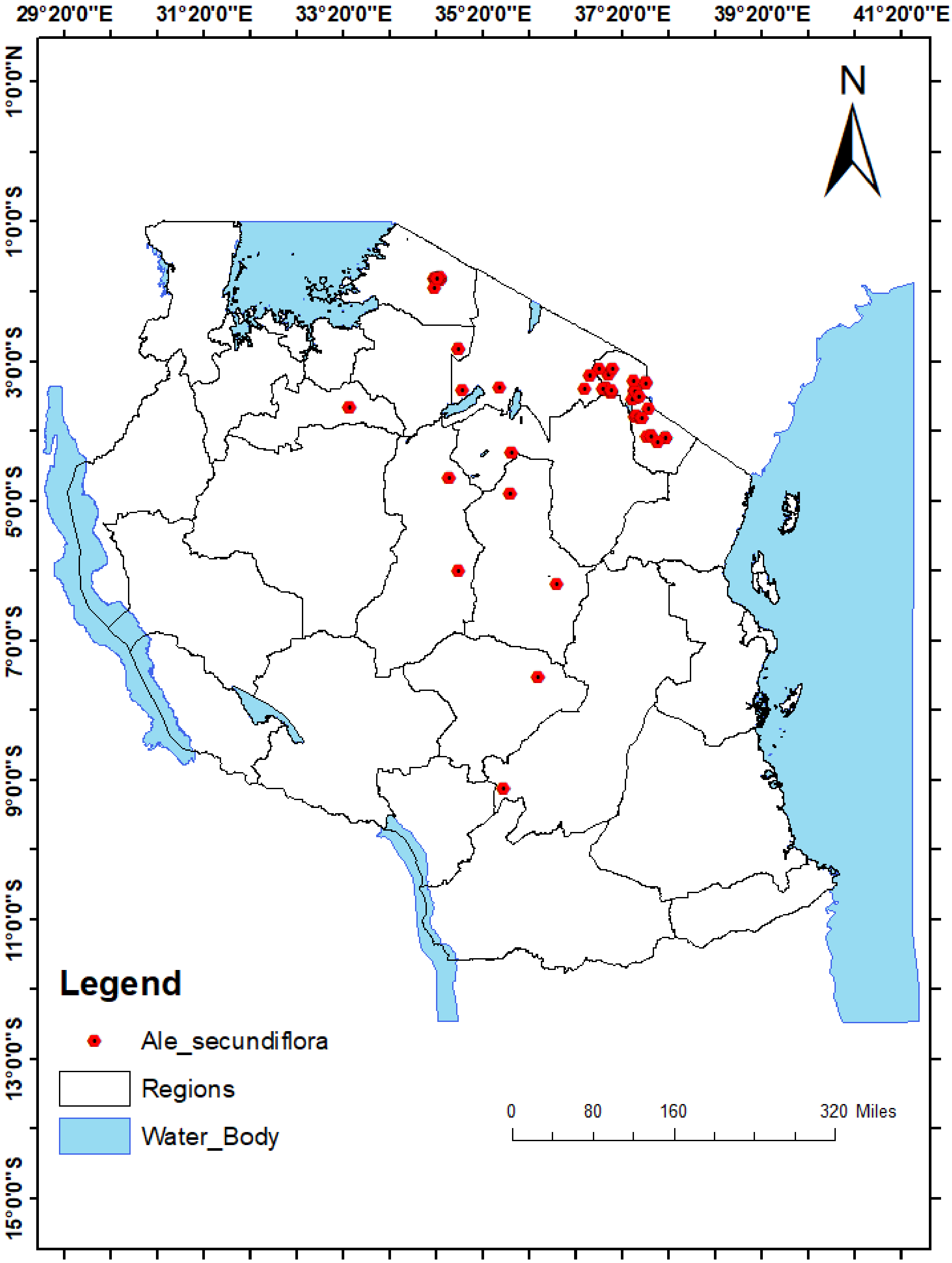

Figure 37: Species locality map for *Aloe secundiflora* in Tanzania

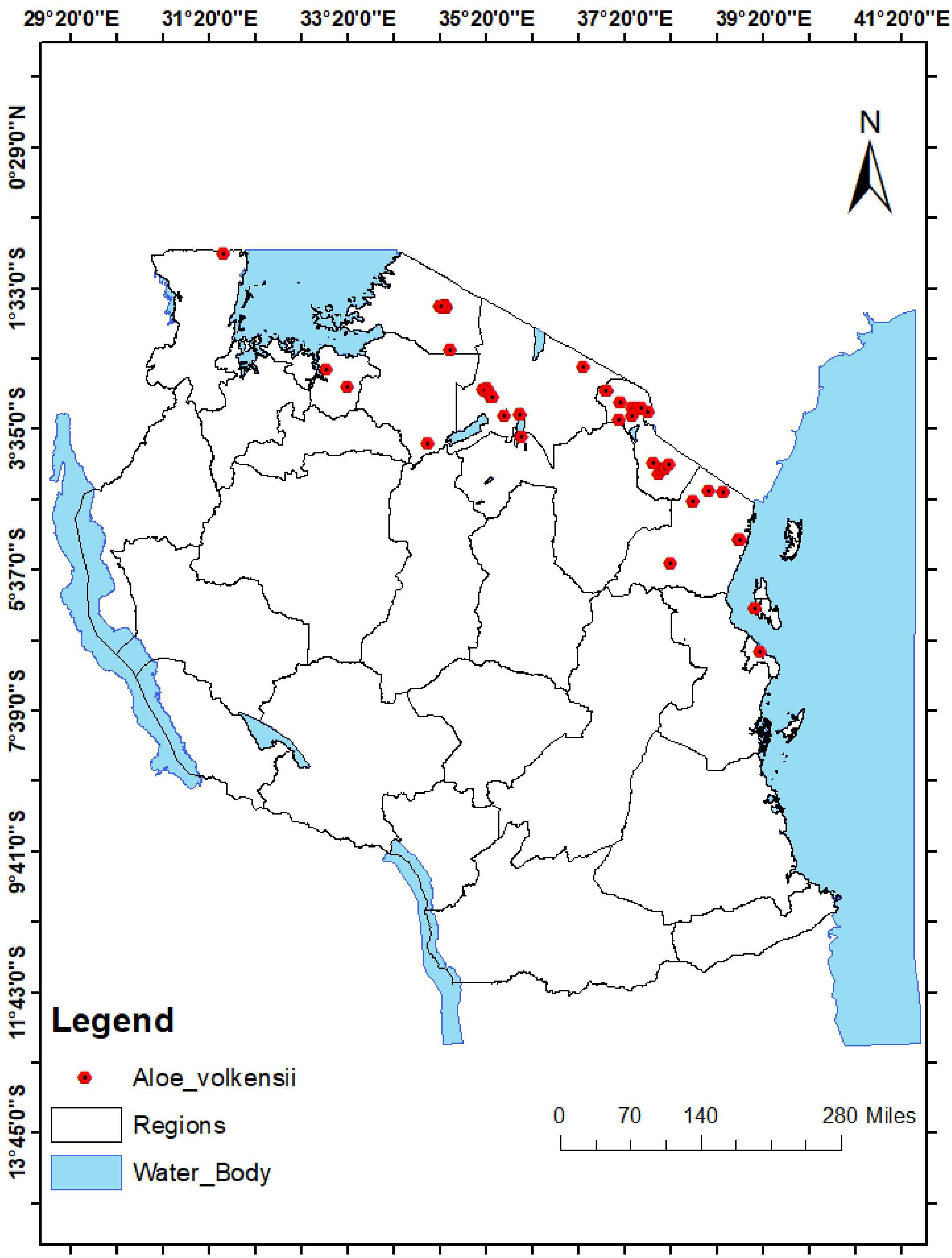

Figure 38: Species locality map for *Aloe volkensii* in Tanzania

RESEARCH OUTPUTS

(i) Published articles

Abihudi, S. A., De Boer, H. J., Mahunnah, R. L. A., & Treydte, A. C. (2019). Ethnobotanical knowledge and threat factors for *Aloe* species in Tanzania. *Ethnobotany Research and Applications*, *18*, 43, 1-28.

Abihudi, S. A., Venkataramana, P. B., Boer, H. J. De, & Treydte, A. C. (2020). Species-specific responses of “Critically Endangered” and “Least Concern” Aloe seed germination to environmental conditions in Tanzania. *Global Ecology and Conservation*, 24, 1-9.

(ii) Poster presentation

ETHNOBOTANICAL KNOWLEDGE AND THE THREAT FACTORS FOR *ALOE* SPECIES IN TANZANIA

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Introduction

The genus *Aloe* has long been known for its use in healthcare and cosmetics (1). In Tanzania, little has been documented on the bio-cu+ltural uses of *Aloe* and some species are at risk of disappearance (2), which might be due to overutilization through humans.

Objectives

1. To assess folk taxonomy and flowering time of *Aloe* species in Tanzania
2. To document the ethnobotanical use of the *Aloe* species in Tanzania.
3. To assess the respondents perceptions on disappearance and conservation activities for *Aloe* species in Tanzania.

Methodology

Study Area

Fig 1. Map of Tanzania with shaded areas showing where interviews were conducted.

Sampling technique

Semi-structured questionnaires (n=237)
22 villages in four regions
(Kilimanjaro, Tanga, Mara, Katavi-Rukwa).

Analysis

Relationship

- Mann–Whitney U-tests-Ethnobotanical knowledge vs age groups and gender.
- Kruskal Wallis Test-Perception on factors affecting *Aloe* vs the number of individuals *Aloe* used.
- Mann–Whitney U-test- age vs perception on possible disappearance

Result

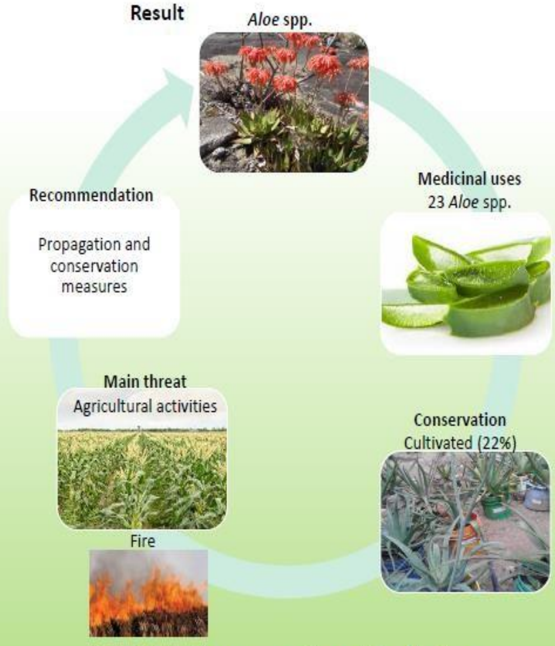

Fig 2. Medicinal uses, conservation and threat to *Aloe* spp.

- Morphological features were used to characterize *Aloe* species.
- Most of the morphologically similar species were considered to be the same species
- The flowering periods of *Aloe* spp. followed the rainy season.
- The mostly used *Aloe* were *A. lateritia, A. duckeri* Christian and *A. secundiflora*
- The regions with a high diversity of *Aloe* species had multiple uses of *Aloe* compared to the regions with low diversity of *Aloe* species.
- Aloes were mostly used to treat malaria (20%), chicken Newcastle disease (19%), and stomachache (7%), as well as hernia and typhoid.
- The respondents' (87%) perceived *Aloe* to be decline in the wild.

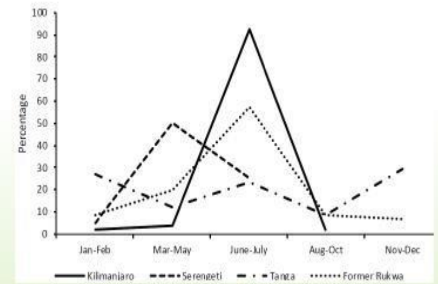

Fig 3. *Aloe* flowering times reported by the study respondents (n=237) in the four study regions.

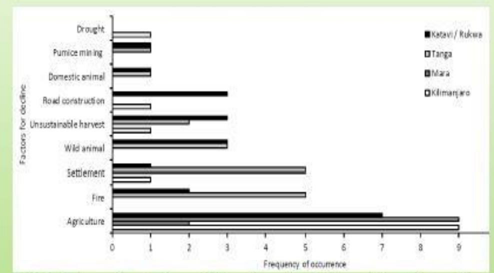

Fig 4. Factors for decline of *Aloe* from field observations from all four study regions

Conclusion

- Need for pharmacological studies to investigate chemical compounds and extracts for the potential clinical and commercial application.
- In-situ and ex-situ conservation of *Aloe* species

Acknowledgments

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References

1. Ribeiro A, Estanqueiro M, Oliveira M, Sousa Lobo J. Main Benefits and Applicability of Plant Extracts in Skin Care Products. Cosmetics [Internet]. 2015;2(2):48–65. Available from: http://www.mdpi.com/2079-9284/2/2/48/

Poster Presentation 2

SPECIES-SPECIFIC RESPONSES OF CRITICALLY ENDANGERED AND LEAST CONCERN *ALOE* SEED GERMINATION TO ENVIRONMENTAL CONDITIONS IN TANZANIA

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Introduction

The genus *Aloe* has been known for its use in healthcare and cosmetics. However, the genus is threatened by anthropogenic activities (1), trade, and the effects of climate change (2) and little is known on seed biology. Understanding the germination behavior of Aloe species will help in the conservation of genus.

Methodology

Five Tanzanian *Aloe* species were used for this study, two critically endangered species, i.e., *A. pembana* and *A. boscawenii,* and three species of least concern, i.e., *A. lateritia, A. secundiflora* and *A. volkensii*.

We evaluated the effects of

- Light condition
- Scarification
- Temperature variation
- Growth media
- Different concentrations of salt and KNO_3

Data Analysis

We used Origin Pro 2015 statistical package to evaluate

- Final Germination Percentage (FGP)
- Main effect of treatment using two-factorial ANOVA.
- Treatment and species comparison using Tukey's post hoc tests

Result

There was a significant difference in the germination of *Aloe* seeds across the main effects of environmental factors and *Aloe* species.

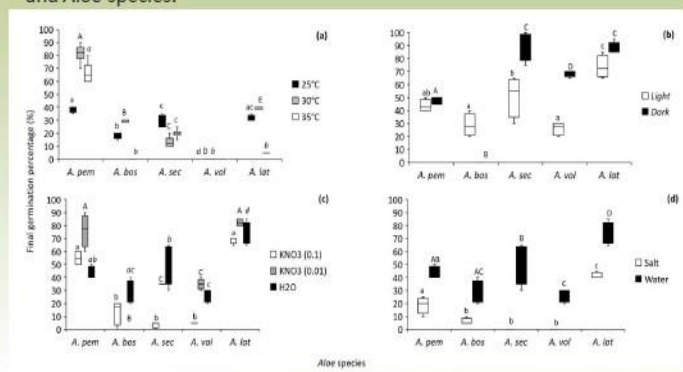

Fig 1. Final Germination percentage of *Aloe* seeds grown at different (A) temperatures, (B) light condition, (C) potassium nitrate concentration and (D) salinity.

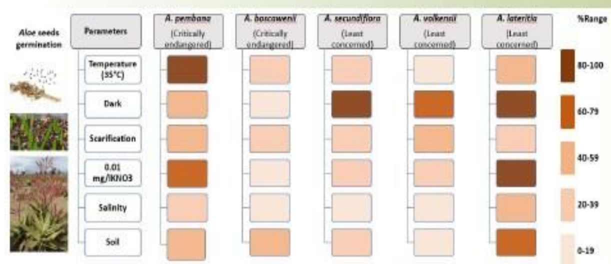

Fig 2. Final germination percentage of *Aloe* seeds across different parameters

Summary

- The optimum temperature for germination is 30 °C
- Darkness stimulates germination (except for *A. boscawenii*).
- *Aloe* seeds germinated well when un-scarified.
- Low KNO_3 concentrations (0.01 mg/l) favored germination.
- For coastal Aloe, seed viability can be improved by recovering Aloe from salt water
- Initiation of sprouts were slightly higher on filter paper water medium compare to the soil medium.

Conclusion and Recommendation

- The *Aloe* species showed species-specific responses to different treatments, which reflected their current Red List status, except for *A. pembana*.
- Other techniques such in vitro culture can be applied for Aloe species with little FGP such *as A. boscawenii*.

Acknowledgments

We are grateful to all district authorities for permission and CREATES through the World Bank for financial support

References

1. CITES. (2003). *Review of significant trade in East African Aloes. CITES. PC14 Do.9.2.2. Annex*
2. Abihudi, S. A., Boer, H. J. De, Rogasian, L. A., & Treydte, A. C. (2019). Ethnobotanical knowledge and threat factors for Aloe species in Tanzania. *Ethnobotany Research & Applications*, 18(43), 1–28.