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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

Arusha, Tanzania

September, 2021

#### 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<sub>3</sub> 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 rediscovered 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<sub>3</sub> 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 speciesspecific 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.

Siri Abihudi

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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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#### ACKNOWLEDGEMENTS

First and foremost, I'm deeply grateful to God for his many blessings, strength and sustenance throughout the mountains and valleys of my academic life.

I want to express my most profound appreciations to my supervisors, Prof. Anna C. Treydte, Prof. Hugo J. De Boer and Prof. Rogasian L. A. Mahunnah, for their guidance, support and general insights in my research project. It has been an inspiring experience for me, and I have learned a lot from you.

I would also like to extend my deepest gratitude to all district authorities for permission to conduct this research in their areas and the support shown by the ward, village leaders and all field assistants during the survey.

I am grateful to the late Prof. Alfred Mzuka, who insisted that I should considered tissue culture as one of my goals in conservation; I can feel its influence in my current career as a conservationist; may his soul rest in peace.

I am extremely indebted to International Institute of Tropical Agriculture (IITA) members, including Dr. Alan Brown, Ms. Magdalena Kiurugo, Ms. Emiliana Biseko, Ms. Neema Palanjo and Ms. Prudence Masanga. Tissue culture experiment would not have been possible without your support and courage.

I am incredibly grateful to Mr. Marcella Rydmark and Dr. Simeon Mesaki for their quick response during manuscript development. I would also like to extend my deepest gratitude to Prof. Tamera Minnick for reading through my manuscript, Dr. Simeon Mesaki and Mr. Deogratius Kikumi for proofreading my dissertation and Mr. Saidi Alfred for creating Arc GIS maps for my dissertation. Many thanks to the lab technicians; Mr. Silvester Temba and Mr. Damas Mnyang'ali for their support during lab work.

Thanks to my fellow students; Mr. Eric Mtegoa, Mr. Deogratius Kikumi, Mr. Bakari Ramadhani, Mr. Paul Lucas, Mr. Beatus Mwendwa, Mr. Saidi Alfred, Mr. Stanslaus Mtavangu and Ms. Tusekile Alfred to name a few, who we spent a lot of time together at the lab, working tirelessly and encouraging each other. Thank you for being there for me when I needed technical advice or someone to talk to. Thanks to my friends outside NM-AIST community and relatives; Mr. Samweli Salakana, Mr. Meki Mesaki, Ms. Merina Mesaki, Ms. Christina Mesaki, Ms. Vaileth Amani, Mr. Issa Washanje, Ms. Zawadi Chediel, and Mr. Edger Mwesiga to name a few, for the

encouragement and always being there for me in my difficult time. Lastly, I thank the Centre for Research, Agricultural advancement, Teaching Excellence and Sustainability in Food and Nutritional Security (CREATES) for provided financial support through the World Bank as a loan to the Tanzanian government.

#### 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).

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	Naturforschung: Senckenberg Forschungsinstitut und Naturmuseum (FR). Siri
	Abihudi (SA)

# 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
Κ	Royal Botanic Gardens Kew, United Kingdom
Κ	Potassium
km	Kilometer
KNO3	Potassium nitrate

1	Litre
LC	Least Concern
m.a.s.l	Meters Above Sea Level
mg	Milligram
Mg	Magnesium
Min	Minutes
ml	Milliliter
Mn	Manganese
МО	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
Р	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
Ν	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  $\pm$  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

- 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

- 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-19<sup>th</sup> 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 Participant Assessment Project Participants, 2009b).

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<sup>2</sup> for CR < 5000 km<sup>3</sup> for EN and  $< 20\ 000\ \text{km}^3$  for VU) and B2 (AOO< 10 \ \text{km}^2 for CR  $< 500\ \text{km}^2$  for EN and  $< 2000\ \text{km}^2$  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 applied to 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,  $\sigma$  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	of Occupancy	that were	used for
	assessing th	ne current statu	s of the thre	atened Alo	e species in Ta	nzania	

8	1						
Geographic range	<b>Critically Endangered</b>	Vulnerable					
B1. EOO	$< 100 \text{ km}^2$	$< 5000 \text{ km}^2$	$< 20\ 000\ km^2$				
B2. AOO	$< 10 \text{ km}^2$	$< 500 \text{ km}^2$	$< 2000 \text{ km}^2$				

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.

species that were germinated in this study								
IUCN Red	Aloe species	District	Accession	Storage	Flowering			
List Status			locations	time	months			
Critically endangered	A. pembana	Pemba	5°21'S, 39°38'E	2	Nov. March			
	1 hosomumii	Miringo Tongo	4°49'S,					
	A. boscawenii	Mikiliga, Taliga	39°6'E	5				
Least	A lateritia	Handeni Tanga	5°25'S,	1.1				
Concern	<i>11. tutertitu</i>	Hundein, Fungu	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,	14				
		zerengen, muru	34°40′E					

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

#### **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.

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

# 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)

#### **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<sub>3</sub> with the ascorbic acid molybdate method; The soil cation, i.e. Ca<sup>2+</sup>, Mg<sup>2+</sup> and K<sup>+</sup> were extracted using NH<sub>4</sub>C<sub>2</sub>H<sub>3</sub>O<sub>2</sub> 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,  $N \ge 50$ ). 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<sub>3</sub>)<sub>2</sub> 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<sup>2</sup>) 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  $5.8 \pm 0.1$  before autoclaving at  $121^{\circ}$ C for 15 min. The cultured samples were maintained at a temperature of  $25 \pm 2^{\circ}$ 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

				Ta	nzania		
Scientific name	Ende mic	Current IUCN status (all global)	Estimated # of locations	EOO (km <sup>2</sup> )	AOO (km <sup>2</sup> )	Newly proposed status	Threat
<i>Aloe ballyi</i> 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
<i>Aloe bicomitum</i> L. C. Leach	No	NE	2	6490	16	EN B2ab(iii) (National assessment)	Overharvesting
<i>Aloe boscawenii</i> 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
<i>Aloe christianii</i> Reynolds	No	NE	5	82 927	68	EN B2ab(iii) (National assessment)	Land for agriculture
<i>Aloe confusa</i> Engl.	No	NE	2	1265	20	EN B1ab(iv)+2ab(iv) (National assessment)	Restricted population and Floods (at Kifaru river)
<i>Aloe deserti</i> 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	Ende mic	Current IUCN status (all global)	Estimated # of locations	EOO (km <sup>2</sup> )	AOO (km <sup>2</sup> )	Newly proposed status	Threat
<i>Aloe dorotheae</i> 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(i ii,v)	Fire from clearing agricultural land and grazing
<i>Aloe duckeri</i> Christian	No	LC (Richart, 2019a)	> 15	167 960	108	LC (National assessment) CR	Land for agriculture and grazing
<i>Aloe fibrosa</i> Lavranos & L. E. Newton	No	NE	1	65	12	B1ab(iv)+2ab(i) (National assessment)	Restricted population
<i>Aloe flexilifolia</i> 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
<i>Aloe lateritia</i> 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

				Ta	nzania		
Scientific name	Ende mic	Current IUCN status (all global)	Estimated # of locations	EOO (km <sup>2</sup> )	AOO (km <sup>2</sup> )	Newly proposed status	Threat
		Plant Assessment Project Participants, 2009n)					
<i>Aloe mzimbana</i> 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
<i>Aloe parvidens</i> M. G. Gilbert and Sebsebe	No	LC (Weber & Demissew, 2013a)	2	206	12	EN B1ab(iii)+2ab(iii) (National assessment)	Land for agriculture
<i>Aloe rabaiensis</i> 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
<i>Aloe volkensii</i> Engl.	No	LC (Eastern Arc Mountains & Coastal Forests CEPF Plant Assessment Project Participants 2009i)	>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. confusa*. 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 overharvested 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 species* (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<br/>% 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).

Fixed factors	df	Main effect		
		F	р	
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 <sub>3</sub> concentration	2	34.3	< 0.0001	
Species x KNO <sub>3</sub>	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	

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

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 unscarified.

Furthermore, *Aloe* seeds' germination percentage differed significantly across KNO<sub>3</sub> concentrations (Fig. 14) with high (0.1 mg/L) KNO<sub>3</sub> 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<sub>3</sub>) and 0.01 mg/L KNO<sub>3</sub> (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<sub>3</sub> 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* 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<sup>th</sup> and the 10<sup>th</sup> day after culture initiation. Contaminations were mainly due to fungal infections (66%, N = 230) at all concentration levels of NaOCl and Hg(NO<sub>3</sub>)<sub>2</sub> 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<sub>3</sub>)<sub>2</sub> (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<sub>3</sub>)<sub>2</sub> 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  $\alpha$ -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 su	rvive on
average per each soil medium (±SE) during the acclimatization proce	ss in the
screen house. Different letters across rows represent significant di	fferences
according to Tukey's Post Hoc tests (p < 0.05)	

Soil Medium	No. of plantlets survived	Multiplication of plantlets
Sand	4.0 ± 0.6a	$12.0 \pm 1.7$ a
Sawdust	$3.0 \pm 0.6a$	8.3 ± 1.5a
Coco peat	$3.3 \pm 0.3a$	$8.7\pm0.7a$
Sand + Coco peat	$3.7\pm0.7a$	$13.3 \pm 2.3a$
Sand + Sawdust	$3.7 \pm 0.3a$	7.7 ± 0.3ab
Sand + Sawdust + Coco peat	$4.7\pm0.3a$	$18.7 \pm 1.3b$
F	0.64	2.16
Р	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 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 (5<sup>th</sup> day) than in cuttings (11<sup>th</sup> 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.

not observed (absent)								
present	absent	Т	df	р				
1.2±0.5	1.2±0.6	0.0	2	0.974				
$5.4 \pm 0.1$	5.7±0.3	-1.8	2	0.214				
0.9±0.1	$1.0\pm0.1$	-0.8	2	0.507				
$0.1 \pm 0.0$	$0.1 \pm 0.0$	-0.2	2	0.850				
4.0±1.2	$4.5 \pm 0.9$	-1.8	2	0.220				
$1.4{\pm}0.7$	2.1±1.6	-0.3	2	0.767				
$0.0\pm0.0$	$0.1 \pm 0.0$	-0.7	2	0.577				
$0.1 \pm 0.1$	$0.1 \pm 0.0$	-0.3	2	0.768				
$0.1 \pm 0.0$	$1.2{\pm}1.2$	-0.9	2	0.453				
40.3±6.8	67.3±8.3	-4.9	2	0.038*				
9.1±4.0	7.0±1.0	0.6	2	0.623				
278.5±75.6	101.1±21.3	1.9	2	0.203				
$0.7{\pm}0.2$	0.5±0.1	4.5	2	0.046*				
91.7±13.8	62.2±8.2	1.4	2	0.289				
$0.0\pm0.0$	$0.0\pm0.0$	-0.1	2	0.939				
	present $1.2\pm0.5$ $5.4\pm0.1$ $0.9\pm0.1$ $0.1\pm0.0$ $4.0\pm1.2$ $1.4\pm0.7$ $0.0\pm0.0$ $0.1\pm0.1$ $0.1\pm0.1$ $0.1\pm0.0$ $40.3\pm6.8$ $9.1\pm4.0$ $278.5\pm75.6$ $0.7\pm0.2$ $91.7\pm13.8$ $0.0\pm0.0$	presentabsent $1.2\pm0.5$ $1.2\pm0.6$ $5.4\pm0.1$ $5.7\pm0.3$ $0.9\pm0.1$ $1.0\pm0.1$ $0.1\pm0.0$ $0.1\pm0.0$ $4.0\pm1.2$ $4.5\pm0.9$ $1.4\pm0.7$ $2.1\pm1.6$ $0.0\pm0.0$ $0.1\pm0.0$ $0.1\pm0.1$ $0.1\pm0.0$ $0.1\pm0.1$ $0.1\pm0.0$ $0.1\pm0.1$ $0.1\pm0.0$ $0.1\pm0.1$ $0.1\pm0.0$ $0.1\pm0.1$ $0.1\pm0.0$ $0.1\pm0.0$ $1.2\pm1.2$ $40.3\pm6.8$ $67.3\pm8.3$ $9.1\pm4.0$ $7.0\pm1.0$ $278.5\pm75.6$ $101.1\pm21.3$ $0.7\pm0.2$ $0.5\pm0.1$ $91.7\pm13.8$ $62.2\pm8.2$ $0.0\pm0.0$ $0.0\pm0.0$	presentabsent $T$ $1.2\pm0.5$ $1.2\pm0.6$ $0.0$ $5.4\pm0.1$ $5.7\pm0.3$ $-1.8$ $0.9\pm0.1$ $1.0\pm0.1$ $-0.8$ $0.1\pm0.0$ $0.1\pm0.0$ $-0.2$ $4.0\pm1.2$ $4.5\pm0.9$ $-1.8$ $1.4\pm0.7$ $2.1\pm1.6$ $-0.3$ $0.0\pm0.0$ $0.1\pm0.0$ $-0.7$ $0.1\pm0.1$ $0.1\pm0.0$ $-0.7$ $0.1\pm0.1$ $0.1\pm0.0$ $-0.3$ $0.0\pm0.0$ $1.2\pm1.2$ $-0.9$ $40.3\pm6.8$ $67.3\pm8.3$ $-4.9$ $9.1\pm4.0$ $7.0\pm1.0$ $0.6$ $278.5\pm75.6$ $101.1\pm21.3$ $1.9$ $0.7\pm0.2$ $0.5\pm0.1$ $4.5$ $91.7\pm13.8$ $62.2\pm8.2$ $1.4$ $0.0\pm0.0$ $0.0\pm0.0$ $-0.1$	presentabsent $T$ df $1.2\pm0.5$ $1.2\pm0.6$ $0.0$ $2$ $5.4\pm0.1$ $5.7\pm0.3$ $-1.8$ $2$ $0.9\pm0.1$ $1.0\pm0.1$ $-0.8$ $2$ $0.1\pm0.0$ $0.1\pm0.0$ $-0.2$ $2$ $4.0\pm1.2$ $4.5\pm0.9$ $-1.8$ $2$ $1.4\pm0.7$ $2.1\pm1.6$ $-0.3$ $2$ $0.0\pm0.0$ $0.1\pm0.0$ $-0.7$ $2$ $0.1\pm0.1$ $0.1\pm0.0$ $-0.7$ $2$ $0.1\pm0.1$ $0.1\pm0.0$ $-0.3$ $2$ $0.1\pm0.1$ $0.1\pm0.0$ $-0.3$ $2$ $0.1\pm0.0$ $1.2\pm1.2$ $-0.9$ $2$ $40.3\pm6.8$ $67.3\pm8.3$ $-4.9$ $2$ $9.1\pm4.0$ $7.0\pm1.0$ $0.6$ $2$ $278.5\pm75.6$ $101.1\pm21.3$ $1.9$ $2$ $0.7\pm0.2$ $0.5\pm0.1$ $4.5$ $2$ $91.7\pm13.8$ $62.2\pm8.2$ $1.4$ $2$ $0.0\pm0.0$ $0.0\pm0.0$ $-0.1$ $2$				

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

\* 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 17<sup>th</sup> 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).

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 preferred for 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).

	Human ailments														Animal ailments						
Scientific name	Urology	Skeleto- muscular	Respiratory	Poisons	Parasites/met azoan	Nervous system	Metabolic issue	Infections	Gastrointestinal	Gynecology	Ophthalmic	Food	Dermatologic	Cardiovascular	Cancer	Antidote	Others	Chicken (Newcastle)	Cows	Others	Non-medicine
A. ballyi		γ	N					γ	N			Ν		γ			d,				
A. bicomitum								N	N					γ		λ		N			
A. chabaudii						V		V	√, e, b	e	b			V			b	N			
A. christianii		V	V		N	N		V	N	e, b			e	√, e		V		N			
A. deserti	V	d, e	d, e			N	d, e	√, d.e	N			V	N	V				d, e	V	d, e	d
A. dorotheae							λ	N										λ			
A. duckerii		N	V	γ	N	γ		ν	N	V			N	√, b		λ		N			
A. flexilifolia						V	γ		N					N	e		λ	N			
A. lateritia		V	√, d, e	b	√, d, e	√, d, e	V	√, d, e	√d	V	d, e	d	√, d, e	b, e		d	√, b	√, d, e	b	d, e	d, b
A. leptosiphon			V	λ		γ	γ	γ	N				N	γ				γ			
A. macrosiphon					V	N		γ	√, e				N	N			с				
A. massawana					V	N	b		N					N				N			
A. mzimbana					V			N	N	V			N	N				N			
A. parvidens		$\checkmark$						N						γ		γ					
A. rabaiensis		V		b		λ		N	N			N	√c	γ					V		
A. secundiflora	v, d, e	√, d, e	v, b, d, e	b	e	√, b, d, e	√, d, e	√, d e	, √, b, d, e		v, d, e	√, a, d	√, b, d, e	√, b, d, e			N	√, b, d, e	√, d	b, d	b, c, d
A. volkensii subsp. multicalius		N	v, b, d, e	b		N	√, e	V	V		√, e	b, b	e	√, e			V	V	√, d	d	d
A. volkensii subsp. volkensii							V	$\checkmark$	V					V				V	V		
<i>Aloe</i> sp. (SA 45)						N			N									N			
Aloe sp. (SA 53)														N							
Aloe sp. (SA 87)						N															

# Table 9: Different uses of *Aloe* species recorded in this study ( $\sqrt{}$ ) and according to five other studies (a-e) in East Africa

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<sup>2</sup>.

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<sup>2</sup> 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 nonendemic, 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<sup>2</sup> 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<sup>2</sup>), 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  $25^{\circ}$ C and  $30^{\circ}$ C and germination success declined at a higher temperature ( $35^{\circ}$ C) supported by other researchers (Kulkarni *et al.*, 2013). The optimum temperature for germination was reported to be  $30^{\circ}$ C, followed by a lower temperature of  $25^{\circ}$ C. *Aloe* species avoid cold conditions below  $20^{\circ}$ 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  $30^{\circ}$ 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<sub>3</sub> 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<sub>3</sub>. 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<sub>3</sub>)<sub>2</sub> 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<sub>2</sub> (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<sub>3</sub>)<sub>2</sub> 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<sub>3</sub>)<sub>2</sub> resulted from the unavailability of HgCl<sub>2</sub>. 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 ( $25^{\circ}C$  and  $30^{\circ}C$ ) 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.

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# 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
	when exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual),
Extinct in the	throughout its historic range have failed to record an individual when it is known only to survive in cultivation, in
Wild (EW)	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	If the species has been evaluated against the criteria mentioned above but does not qualify. However, it is likely to
(NT)	be eligible for a threatened category soon.
Least Concern	If the species has been evaluated against the criteria mentioned above but does not qualify. The species is
(LC)	abundant and widespread.
Data Deficient	There is insufficient data regarding the species to make a primary or secondary assessment of its risk of extinction
(DD)	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,	Likulakula	Bena	Aloe	Short spotted Aloe
46, 67, 73, 74, 77, 82, 85, 88, 155)	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	Pare	Aloe	Tall Aloe
	vujawa			
Aloe bicomitum L.C.Leach	Tembosha/Itemboshya	Fipa	Aloe	Spotted Aloe from the
(SA-137, 145)				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,	Igaka/Iughaka	Sukuma	Aloe	Green Aloe
138)	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,	Kikoli chekundu	Zigua	Red Aloe	Red with spots
76)				
Aloe duckeri Christian (SA-114, 116,	Ivata or Amavata	Nyika	Aloe	Huge spotted Aloe
117, 118, 122, 124, 126,128, 129, 131,	(plural)	(Sumbawanga)		
134, 140, 142, 148)	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	Sambaa	Aloe with	Hanging on cliff surface
	marefu		long leaves	
	kovongo	Sambaa	Aloe	Aloe with dark leaves
Aloe leptosiphon A.Berger (SA-20, 21, 22, 23,	Katani ya dawa	Sambaa	Medicinal	Spines at the side, bitter
30, 32, 33, 35, 36, 41)			sisal	taste

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

Scientific name	Vernacular name	Tribe	Translation	Informant description
Aloe parvidens M.G.Gilbert & Sebsebe (SA-	Kithapa/Kisapa	Pare	Aloe	Spotted Aloe
171, 174)	Kinabwele	Pare	Aloe	Spotted Aloe
	Kithapa/Kisapa	Pare	Aloe	Spotted Aloe
Aloe secundiflora Engl. (SA-150, 152, 169,	Olsukuroi miguu	Maasai	Aloe with	Short unspotted Aloe
184)	mingi		many legs	
	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	Maasai	Aloe with	Short unspotted Aloe
	mingi		many legs	
	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,	Ekighaka/Kighaka	Kurya	Aloe	Tall with spots when
96, 160, 161, 162, 166, 168, 175, 185, 186)				young
	Kithapa/Kisapa cha	Pare	Aloe	Tall Aloe
	vujawa			
	Olsukuroi mguu	Maasai	Aloe with	Tall Aloe
	mmoja		one leg	
	Kashaza ndefu or	Sambaa	Tall Aloe	Tall Aloe
	Maoza/oza			
	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)	Kikoli	Zigua	Aloe	Red/green Aloe
(SA-45)				
Aloe (Unidentified)	Ekighaka/Kighaka	Kurya	Aloe	Tall Aloe
(SA-53)				

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

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

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

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

Scientific name	Human ailment	Animal ailment	Cosmetic	Local brew	Others	IUCN Red List Status
Aloe		Chicken				
(Unidentified-		(Newcastle				
SA 45)	Hernia, Malaria, Stomach ache, Wounds	disease)				
Aloe		Chicken				
(Unidentified-		(Newcastle				
SA 53)	Malaria	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,		
		2018	Katavi,	Msukumilo, Matai,		
1 duckori	S.Abihudi		Rukwa,	Msanzi, Laela, Namanyele,	MO(2)	
A. auckeri	A.Chawala	2019	Iringa, and	Mpanda, Njombe, Mufindi,	MO(3)	
			Njombe	Ugala River,		
A fibroga	S Abibudi	2019	Vilimonioro	Simba farm in	K (3)	
A. Jibrosa	S.Abiliudi	2018	Kiimanjaro	Engarenairobi ward		
A. flexilifolia	S.Abihudi	2018	Tanga	Soni, Kisiwani village	MO (4)	
				Kisangula, Pongwe,		
				Handei, Magamba,		
				Kwematungutu, Migulu,		
				Shukilai, Migambo,		
				Kifulilo, Kifungiro,		
A. lateritia				Masange, Gale, Kisangula,		
	S.Abihudi	2018	Mara, Kilimanjaro,	Handei, Bangu,	K (51), MO	
				Kwamkono, Kwamagome,	(6)	
			and Langa	Golani, Handeni, Mbamba,		
				Hoza,Kwafivi, Mshizii,		
				Kwamsononi, Soni,		
				Lushoto, Bomole hill,		
				Amani, Rombo, Dodoma,		
				and Mbulu.		
<i>A</i> .				Soni, Kishewa, Magila,		
leptosiphon	S.Abihudi	2018	Tanga	Kwamsononi,	MO (4)	
				Mgombelwa, and Mlalo		

Scientific name	Collector	Year of collection	Region	Locality	Specimens in Western herbaria (# of specimens)
A. macrosiphon	S.Abihudi	2018	Mara	Mara Serengeti NP	
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
- 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 <i>Aloe</i> in your area?
18.	Is it in high demand or low?
19.	How much is the <i>Aloe</i> sold per leave?
	a. 100-500
	b. 600-1000
	c. 1000-1500
	d. Above 1500
20.	What are the threat factors to <i>Aloe</i> spp
21.	When is flowering period for <i>Aloe</i>

22. Please list the *Aloe* species and other information needed in the Table 1.

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

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

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

## Poster Presentation 1



# 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 biocu+ltural uses of Aloe and some species are at risk of disappearance (2), which might be due to overutilization through humans.

#### Objectives

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

#### Methodology



### 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



Recommendation

Propagation and

conservation

measures

Main threat

Agricultural activities

Fire



Medicinal uses

23 Aloe spp.





#### Conclusion

Agricultur

100

90

80 70

50 40

Jan-Feb

Pumice min

Road construc ustainable han Wild ani Sattlerr

Kilmaniaro

Mar-May

June-July

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

the four study regions.

---- Serengeti - - Tanza ----- Former Rukwa

Aug-Oct

Nov-Dec

Katavi / Rukura

**O**Tanga

•Mara

**D**Klimaniaro

- 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

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

#### 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.mdoj.com/2079-9284/2/2/8/

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

. The mostly used Aloe were A. lateritia, A. duckeri Christian and A. secundiflora

Most of the morphologically similar species were considered to be the same species

The regions with a high diversity of Aloe species had multiple uses of Aloe compared to

Aloes were mostly used to treat malaria (20%), chicken Newcastle disease (19%), and

Morphological features were used to characterize Aloe species.

The flowering periods of Aloe spp. followed the rainy season.

The respondents' (87%) perceived Aloe to be decline in the wild.

the regions with low diversity of Aloe species.

stomachache (7%), as well as hernia and typhoid.

## **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. poscawenii, 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<sub>3</sub>

## 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



There was a significant difference in the germination

of Aloe seeds across the main effects of environmental factors

Result

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<sub>3</sub> 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
- 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.

