DOCUMENTATION AND IDENTIFICATION OF MEDICINAL PLANTS TRADED IN TANZANIA BY MEANS OF DNA BARCODING

Siri Abihudi, B.Env.Sci

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DOCUMENTATION AND IDENTIFICATION OF MEDICINAL PLANTS TRADED IN TANZANIA BY MEANS OF DNA BARCODING

By

Siri Abihudi, B.Env.Sci

A dissertation Submitted in (partial) Fulfillment of the Requirements for the Degree of Master of Science (Traditional Medicine Development) of Muhimbili University of Health and Allied Sciences

Muhimbili University of Health and Allied Sciences
October, 2014

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled "**Documentation and identification of medicinal plants trade in Tanzania by means of DNA bar-coding**" in partial fulfilment of the requirements for the degree of Master of Science in Traditional Medicine Development of the Muhimbili University of Health and Allied Sciences.

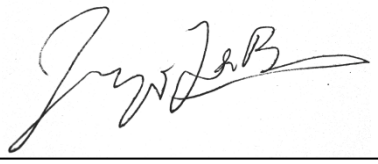
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Dr Nicolao J. Otieno

(Supervisor)

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Date



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Dr Hugo J. De Boer

(Supervisor)

_____

Date

DECLARATION AND COPYRIGHT

I, **Siri Abihudi**, declare that this **dissertation** is my original work. It is being submitted in partial fulfilment for the Master of Science in Traditional Medicine Development at the Muhimbili University of Health and Allied Sciences. It has never been submitted before for any degree award in any other university.

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ABSTRACT

Background

Tanzania is rich in biodiversity and home to a lot of endemic plants that are used in treatment of diseases. The country continues to lose plant biodiversity through trade of medicinal plants. The medicinal plants are traded in the forms ranging from: raw state (leaves, bark and roots) to unprocessed fragmented materials (chips and slices), and semi-processed materials (powders, extracts and teas) which makes conventional identification challenging. In this study the use of DNA barcoding was trialled.

Objectives

The aim of this study was to identify medicinal plants that are trade in Tanzania by means of DNA barcoding.

Methodology

A total of 78 respondents were obtained using the "snowball" technique and personal communication. Data collected was done through local market survey using semi-structured questionnaire, botanical collection, literatures, and laboratory works. Microsoft Excel ware used to structure frequencies, percentage and figures.

Result

There is trade in medicinal plants in Dar-es-Salaam and Tanga regions. Most of these medicinal plants are collected from Coast, Tanga and Arusha regions. The trade is much on most important medicinal plants in the markets which include *Zanthoxylum chalybeum* Engl., *Zanha africana* (Radlk.) Exell, *Cassia abbreviata* Oliv., *Albizia anthelmintica* Brongn., *Hymenaea verrucosa* Gaertn., *Rapanaea melanocephalus and Ximenia aegyptiaca* L.. Threat to medicinal plants have been much on most important plants in the market and those affected through debarking which include: *Albizia anthelmintica* in Bwawa village and *Afzelia quanzensis.*, *Hymenaea verrucosa.* and *Pterocarpus bussei.* Human activities have also impacted on the availability of medicinal plants.

Molecular identification of medicinal plants was successful in identification 52% (n=27) of sample sequenced. There is high possibility of contamination of the sample sequenced especial to those that gave wrong identification.

Conclusion and recommendation

There is high domestic trade in medicinal plants. DNA barcoding proved to be successful in identifying sample sequenced. This method can be applied in controlling illegal trade of endangered medicinal plants in Tanzania.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ATPS	African Technology Policy Studies
BLAST	Basic Local Alignment Search Tool
BSA	Bovine Serum Albumin
CBD	Convention on Biological Diversity
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CO1	Mitochondrial Cytochrome C Oxidase 1
CTAB	Cetyltrimethylammonium Bromide
DNA	Deoxyribonucleic Acid
dNTP	deoxyribonucleate Triphosphates
DRC	Democratic Republic of Congo
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
FAO	Food and Agriculture Organization
FBD	Forestry and Beekeeping Division
HIV	Human Immunodeficiency Virus
INR	Institute of Natural Resource

ITM	Institute of Traditional Medicine
ITS	Internal Transcribed Spacer
IUCN	International Union for Conservation of Nature
MATK	Megakaryocyte-Associated Tyrosine Kinase
MgCl	Magnesium Chloride
MP	Medicinal Plant
MUHAS	Muhimbili University of Health and Allied Sciences
NAI	Nordic Africa Institute
NCBI	National Center for Biotechnology Information
NEAP	National Environmental Action Plan
NEAP	Netional Environmental Action Plan
NGOs	Non Government Organisations
NH4	Ammonium Ion
nrITS	nuclear ribosomal Internal Transcribed Spacer
PCR	Polymerase Chain Reaction
PROTA	Plant Resources of Tropical Africa

CHAPTER ONE

1.1 INTRODUCTION

Tanzania is internationally known to be rich in biodiversity, with over 12,000 plant species (Kuete, 2013). The Eastern Arc Mountains are home to endemic plants and a number of them are used in traditional medicine practices (Mwaseba *et al.,* 2000; Kuete, 2013), 1,100 of which are endemic are located mainly in the coastal regions (Kuete, 2013).

Plants are used in traditional medicine than other materials (FAO, 1997; WHO, 2007; Abihudi, 2012). They are also used for other purposes like timber, fuel wood and charcoal. This exposes wild medicinal plants to over exploitation and even possible threat of extinction.

In many countries traditional medicine is recognized and practiced in parallel with modern medicine (Tabuti *et al.,* 2003). Wild harvesting of medicinal plants by traditional healers has significant impacts. This is because the healers do not practice sustainable utilization, which threatens the continued availability of the medicinal plants in the wild. For example, the export of *Osyris lanceolata* Hochst. and Steud. (*sandalwood*) by some traders indicates the likely extinction of the species resource especially in the Shengena forest of Southern Pare Mountains in Tanzania (Kuete, 2013).

Tanzania is a party to CITES (November, 1979), CBD (March, 1996) and the regional Lusaka Agreement (May, 1982) on cooperative enforcement operations, directed at illegal trade in wild fauna and flora (UNEP, 2007). The Forest Act of 2002 is another law designed for safeguarding biodiversity. However there is weak enforcement and as a result Tanzania continues to lose plant biodiversity through illegal trade of medicinal plants (Kuete, 2013). The commercial cultivation of medicinal plants is not one of the government's priorities (FAO, 2005; Kuete, 2013).

Plants are traded in a variety of forms, ranging from raw state (leaves, bark and roots) to unprocessed fragmented materials (chips and slices), and semi-processed materials (powders, extracts and teas) (INR, 2003; Moeng and Potgieter, 2011) to processed products like soaps, lotions and tinctures. This makes the identification of wild-harvested medicinal plants in trade to be complicated, hence the need of DNA barcode technique. Sometimes they are prepared in combination of either two or three plants, depending on the ailment (Tabuti *et al.,* 2003; Moeng and Potgieter, 2011).

DNA barcoding, refers to the generation and analysis of DNA sequences from biological samples (Segersäll, 2011). It is designed to provide rapid, accurate, and automatable species identifications by using short, standardized gene regions as internal species tags (Hebert and Gregory, 2005).

Identifications can be made with small amounts of tissue from sterile, juvenile or fragmentary materials (Janzen, 2009. It enables specimen identifications when diagnostic characters are difficult to interpret or do not exist (Janzen, 2009; Segersäll, 2011) and harmonizes the work being performed by botanists, zoologists, and microbiologists. It makes use of short (<1000 bp), agreed-upon regions of the genome (a "barcode") that evolve fast enough to differ between closely related species. It is done by comparing the DNA sequences obtained in the lab to reference sequences from international databases. It makes the Linnaean taxonomic system more accessible, with benefits to ecologists, conservationists, and the diversity agencies (Veldman, 2014).

DNA barcoding has immense conservation application, particularly in the enforcement of bans on trafficking of protected species. Barcoding could allow for relatively rapid and accurate identification by non-specialists of listed taxa at customs checkpoints. In addition, the identification of morphologically deficient or incomplete specimens can also be undertaken (e.g., powders) (Little, 2007)

1.2 Literature Review

1.2.1 Medicinal Plants Trade

The greater part of the world biodiversity is found in the tropics (Etkin, 1998). More than 2500 of the plants that are in the wild are medicine (ITM, 2012). Local communities in the tropics have an intimate relationship with nature and posses abundant indigenous knowledge on the use of medicinal plants (Etkin, 1998).

Plants were the first material used to treat illness and diseases. About 50,000 to 70,000 plants species are believed to be used medicinally worldwide of which about 3,000 species of medicinal plants are traded internationally (Gillespie, 2011). The largest importer of medicinal plants in volume is the USA which account for (75%) (Lange, 2004). Japan, Germany and Hong-Kong take 95% of the wild ginseng trade (Lange, 2004; Gillespie, 2011; Herrero and Martin, 2012). The two main species of ginseng used for medicinal purposes are Asian ginseng *(Panax ginseng)* and American ginseng *(Panax quinquefolius)* (Lange, 2004).

The global use of medicinal plants is increasing due to high demand from the consumers (INR, 2003; Chang *et al.,* 2011; Moeng and Potgieter, 2011; Hong, 2012; Herrero and Martin, 2012; Nahashon, 2013). WHO stated that, eighty percent (80%) of the world's population depends on different plants and herbs for treating ailments (WHO, 2011). South African traditional healers indicated an increase in the number of patients they had attended to over the previous five years and 81% expected a further increase in the coming five years (Dold and Cocks, 2002). Medicinal plants provide an important contribution to health, local economies, and cultural integrity and ultimately the well being of people (INR, 2003; Hamilton, 2003; Osemeobo, 2010).

Wild plants form a significant component of modern healthcare practices. Pharmaceutical production is consuming a greater share of medicinal plants harvested from the wild for some species (INR, 2003; Laird, 2007; Mulliken, 2008; Herrero and Martin, 2012). Compounds

such as reserpine from Snakeroot *Rauvolfia serpentina* and paclitaxel from Himalayan Yew, Taxus wallichiana have important pharmaceutical uses in Europe and North America (INR, 2003; Mulliken, 2008; Herrero and Martin, 2012). Some medicinal plant species are also in demand for their aromatic properties. Thus the use of the oil of Jatamansi, *Nardostachys grandiflora*, for example, appears in written texts dating back over a thousand years. Some Tanzanian traders export essential oils such as Sandalwood oil, Eucalyptus oil, Rosemary oil and Ocimum oil (Kuete, 2013).

1.2.2 Threat to medicinal plants

Wild plant species form the foundation of healthcare practices throughout the world (Mulliken, 2008). Eighty percent (80%) of the medicinal plants for raw drug trade in India and other south Asian countries are predominantly collected from the wild, by local farmers or collectors (Sriramaa *et al.,* 2010). The intensity and frequency at which these resources are collected, as well as the techniques used, is unsustainable (Osemeobo, 2010; Chang *et al.,* 2011; Kuete. 2013).

Most of medicinal plants used are herbs (Stepp, 2001). Ecological and biochemical evidence may help to explain the preponderance of herbs in traditional medicines. Herbs and shrubs are primarily preferred and used for their roots. In contrast, trees are mostly used for their bark, with a few species being utilized for their leaves (Moeng and Potgieter, 2011).

In most cases, one plant part is used to treat more than one condition, while several plant parts can be used to treat the same condition. One species can be used for different ailments depending on its administration and application. Most of the related species are used to treat similar ailments. In South Africa, roots, leaves and barks are generally used to treat people suffering from common ailments such as colds, flu and cuts and to rare ailments such as HIV/AIDS, gynaecological problems and ulcers. Roots are mainly used for the treatment of female infertility and male impotence. Leaves are primarily used to treat colds and flu, while bark is mostly used to treat wounds (Moeng and Potgieter, 2011).

The supply and trade of medicinal plants is gradually causing ecosystem instability (INR, 2003; Laird, 2007; Mulliken, 2008; Osemeobo, 2010; Moeng and Potgieter, 2011; Herrero and Martin, 2012) genetic erosion and loss of population (Herrero and Martin, 2012; Nahashon, 2013). The forests are being harvested beyond their annual yields thereby devastating the resource base (Mwaseba *et al.*,, 2000; Laird, 2007; Osemeobo, 2010). In India, because market-driven harvesting did not pay attention to conservation the resource base of medicinal plants has been adversely affected (Osemeobo, 2010). It is believed that, two thirds of the world's plant species are in danger of extinction during the course of the 21st century. In 2009, it was estimated that up to 15,000 medicinal plants were under direct threat in the wild due to trade (Gillespie, 2011). In South Africa the species of *Brackenridgea zanquebarica* and *Warburgia salutaris* are threatened by trade (Moeng and Potgieter, 2011). The harvest of leaves, seeds or fruits have less impact on individual plant than the harvest of bark, root, bulb or the whole plant (Laird, 2007).

Trade in medicinal plants takes place mostly through the informal sector. For example in Nigeria, there are no harvesting standards and also the volumes harvested per species are unknown (Osemeobo, 2010). The same situation applies to Tanzania with the likely extinction of the Sandalwood species in the Shengena forest of the southern Pare Mountains (Kuete, 2013). Tanzania species of *Cassia abbreviata, Zanthoxylum usambarense, Zanthoxylum chalybeum, Myrsine africana, Milicia excelsa* and *Prunus africana* (Nahashon, 2013) are threatened because of up-rooting and/or ring-barking.

Asian governments, non-governmental organizations and some private sector have begun to invest in cultivation of certain species to meet international demand and to ensure collection is done in sustainably and accordance with CITES. Yet the evaluation of status found that the implementation of collection and trade controls was generally low, illegal trade is still present (Mulliken, 2008).

According to CITES, the legal trade in wildlife in world was estimated in 2010 to be around $160 billion per year. The illegal trade in species is believed to be $10 and $20 billion (Gillespie, 2011). Demand for plant-derived medicines has created a trade in indigenous plants in South Africa; currently estimated to be worth approximately R270 million a year (Dold and Cocks, 2002). The full extent of illegal traded is unknown. Parties to CITES continue setting the secretariat task to ascertain the exact extent of traded Medicinal plants (Gillespie, 2011).

1.2.3 Medicinal plants identification through DNA Barcoding

The term "DNA barcoding" has been coined to describe the use of a short gene sequence from a standardized region of the genome as a tool for species identification (Hebert and Gregory, 2005). DNA is the carrier of genetic information in most organisms. The DNA is located in the nucleus, and to some extent in the mitochondria and chloroplasts, of eukaryotic cells and in the cytoplasm of prokaryotic cells.

Identification of materials using DNA sequences instead of morphological features is not a new technology. Researchers have been using sequence based ''DNA fingerprinting'' to identify specimens when the only available material is of inadequate quality to make a morphological based determination (Little, 2007).

DNA barcoding of plants presents a number of challenges compared to DNA barcoding in many animal clades. The CO1 marker is present in both animal and plants but in plants it evolves too slowly to have any use for species identification (Kool *et al.,* 2012). Allopolyploids make it hard to make reliable barcode identifications because chloroplast markers are only inherited from one of the parent species (Cowan, 2006; Kool *et al.*, 2012).

From the two chosen core plant DNA barcode regions *rbc* L and *mat* K plus two supplementary regions *trn* H–*psb* A and internal transcribed spacer (ITS1) (or ITS2) have demonstrated reasonable levels of species discrimination in both floristic and taxonomically focused studies (Blomstergren, 2003).

Matk is one of the most rapidly evolving plastid coding regions and it consistently showed high levels of discrimination among angiosperm (Janzen, 2009). The rapidly evolving internal transcribed spacers of nuclear ribosomal DNA also represent a useful supplementary barcode in taxonomic groups in which direct sequencing of this locus is possible

Molecular identification has been found to be successful in most study. The most successful barcode region was ITS (83% correct matches), followed by *mat*K (50% correct matches) in the study done by Syme *et al.,* (2013). Cluster analysis using the ITS2 barcode in Selaginellaceae was basically consistent with traditional plant morphology (GU *et al.,* 2013). ITS exhibits high levels of within species and even within-individual sequence differentiation (Kress and Erickson, 2007).

A two-marker combination of plastid rbcL and matK has previously been recommended as the core plant barcode, to be supplemented with additional markers such as plastid trnH–psbA and nuclear ribosomal internal transcribed spacer (ITS) (Janzen, 2011).

Molecular analysis on plants will add a new dimension to systematic and evolutionary studies of plant diversity (Sang, 2002). This will be through identifying plants and sketching the phylogenetic tree for all plants on earth.

1.3 Problem statement

The demands for medicinal plants has increased in the world due to incurable diseases like AIDS and cancer (Mwaseba *et al.,* 2000; INR, 2003; Chang *et al.,* 2011; Moeng and Potgieter, 2011; Hong, 2012; Herrero and Martin, 2012; Nahashon, 2013). The trade in medicinal plants is unmonitored and often not done sustainably (Laird, 2007; Mulliken, 2008; Osemeobo, 2010; Moeng and Potgieter, 2011; Herrero and Martin, 2012; Kuete, 2013). For example roots of medicinal plant species can be destructively harvested and tree trunks can be circularly de-barked, causing death of the species (Nahashon, 2013; de Boer, 2005). Scientists agree that the yearly rate of extinction has increased from about one species per million to 100-1,000 per

million. This means that thousands of plants and animals are lost each year, most of which have not yet been identified (CSHL, 2012). Until now, biological specimens were identified using Linnaeus formalized biological classification system of binomial nomenclature that assigns each organism a genus and species name. Morphological features like the shape, size and color of body parts are used in this system (CSHL, 2012; Fazekas *et al.,* 2012). If a specimen is damaged or is in an immature stage of development, even specialists may be unable to make identifications (Fazekas *et al.,* 2012) like some forms of traded biological materials such as powders, dried barks and roots (Kool, 2012). Closely related species and presence of admixtures in medicinal plants traded can't be controlled by the Linnaeus methods of classification, hence the need of DNA barcoding (de Boer, 2014). At the same time the status and trade of medicinal plants is very poorly studied, situations explained by the vast size and remoteness of the species' habitats (Mulliken, 2008) hence this attempt to document and identify medicinal plants that are in danger or threatened with extinction.

1.4 Justification of the study

This study will raise the awareness about the need of conserving specific medicinal plants that are endangered through trade to the vendors and other stakeholders of plants resource. This will be possible by coming up with feasible conservation strategies both in-situ and ex-situ. Ex-situ conservation of medicinal plants by vendors and traditional healers will maintain the demand of medicinal plants that are important in the trade.

DNA barcoding method can serve as a new tool in the taxonomist's toolbox as well as being a novel device for non-experts who need a quick identification. DNA barcoding method can be used to identify the presence of admixtures in medicinal plants trade. It will help in distinguishing between species that are closely related and facilitate the control of both legal and illegal trade in medicinal plant species.

The Institute of Traditional Medicine will benefit with new knowledge of DNA barcoding and can incorporate this method in its curriculum. ITM is more close to the traditional healers, so it

will benefit the traditional healers and the Institute in the identification of the medicinal plants that are in the market.

This study will help in determining trade implications to the important medicinal plants in the market which in turn will contribute to one of the goals of CBD, CITES and Lusaka agreement of identifying threatened medicinal plants and monitoring of national and international trade of medicinal plants.

1.5 Research Questions

i. What are medicinal plants that are traded in Dar-es-salaam and Tanga regions of Tanzania?
ii. Are there specific regions for collecting medicinal plants?
iii. What is the status of Medicinal plants in their collection cites?
iv. Can DNA barcoding be a suitable tool in identify crept form of market samples?

1.6 Objectives

1.6.1 Main objective

The main objective of this study was to assess trade trend in medicinal plants that are traded and to identify threatened medicinal plants by means of DNA barcoding for conservation purposes.

1.6.2 Specific objectives

1. To identify traded medicinal plants from vendors in Dar-es-Salaam and Tanga regions of Tanzania.
2. To determine market implications to the availability of important medicinal plants in trade.
3. To use DNA barcoding in identifying medicinal samples not identified by conventional methods.

1.7 Limitations of the study and mitigations

i. It was difficult to find the vendors of medicinal plants; that why sampling techniques snowball was applied**.** A snowball sampling method was used because vendor's population is not documented and it was difficult to locate them. Data was collected on few vendors and then they were requested to provide ethno information that would help to locate other vendors they know in Dar-es-Salaam and Tanga.

ii. Language was also a barrier during interviewing the vendors of medicinal plants, especially with vendors who belong to Masaai tribe. The translation from those who know Swahili helped to get the information; and where the researcher failed to get a translator, the vendors was not interviewed.

iii. Some medicinal plants were hard to find in Bwawa village. It took us more than two hours looking for *Rapanaea melanocephalus* (*Mpaja)* and (*Kasera)* without success. These plants can only be found in a very small part of the forest. These plants were among the priority plants in our list. Collectors assisted to find those rare plants were given more money for the time wasted.

CHAPTER TWO

2. RESEARCH METHODOLOGY

2.1 Study area

The study was carried out in Dar-es-salaam city and in Tanga region from August 2013 to mid-April 2014. Dar es Salaam, located on the eastern coast of Tanzania, lies between latitude 6.45^{o}S and 7.25^{o}S, and longitude 39^{o} E and 39.55^{o} E (Google map). Dar es Salaam is the major commercial, administrative and industrial city, and the largest urban centre in Tanzania. Tanga region is situated at the extreme north-eastern corner of Tanzania between 4^{o} and 6^{o} degrees below the Equator and 37^{o} -39^{o} 10'degrees (Google map) east of the Greenwich meridian**.**

Dar-es-Salaam and Tanga regions were chosen because in the case of the former there is a market centre for medicinal plants at Kariakoo area and the later region is near the floristically diverse Usambara Mountains (McMillen, 2008). The Tanga region is also famous in the country for traditional knowledge for curing physical, cultural, and psychological ailments (Wolfensohn, 2004).

2.2 Research techniques and data collection

2.2.1 Research technique

Permission to conduct this study was sought from ethics and clearance committee of MUHAS. The study targeted all vendors and healers who trade medicinal plants in Ilala, Kinondoni and Temeke districts in Dar-es-Salaam region and Muheza and Tanga district in Tanga region. A total of 78 vendors (Table 1) were sampled using the “snowball” sampling technique because there was no clear information about where they could be found. Enough information and consent form (Appendix 1 and 2) was given and filled by the vendors before they were interviewed. Data was collected using a semi structured questionnaire (Appendix 3 and 4) and was administered to respondents.

It was assumed that the population of vendors is normal distributed (50%). This is because; there was no previous proportional of medicinal plants vendors in Dar-es-Salaam and Tanga region. The sample was calculated according to the formula

$$N = \frac{1.96^2 P(1-P)}{d^2}$$

Where

P=50% of vendors, normal distributed.

d= degree of accuracy taken at 0.011 for 0.95 confidence interval.

2.2.2 Market survey

Total of 306 both semi processed (powdered and packed) and unprocessed (roots and barks) plants samples were bought to assist in molecular identification. Chain of medicinal plants trade was also documented to identify how the medicinal plants trade is carried. The researcher visited 6 check points for natural resources (Table 1). Natural resources officers were visited in order to find out the amount of medicinal plants and plants product transported in and out of the regions. The check points visited were: Pugu road, Kilwa road, Dar-es Salaam seaport and Tanga seaport.

Table 1: Frequency of respondents who participated in the study

Respondents	**Frequency**
Medicinal plant vendors	78
Check points officers	6
Total	**84**

2.2.3 Field survey

Mostly mentioned medicinal plants and those that are in IUCN red list of threatened species were prioritized as a starting point to assess conservation of medicinal plants that are affected by marketing/trade. Permission to collect Medicinal plants samples was obtained from the Forest and Agricultural offices in Coast region and the regional secretariat of Coast and Tanga

regions. Vendors who mentioned medicinal plants that ranked as most mentioned were asked to assist in voucher specimens' collection. The collection of medicinal plants voucher specimens took place in Chalinze, Msoga, and Ubenazomozi wards in Bagamoyo district and Ruvu Chini ward, Kibaha district in Coastal region. In Chalinze ward the villages were Kitonga, Bwawa and Mboga. In Tanga region, collection of medicinal plants took place in Makoe, Hemtoe and Kihitu villages in Mlalo ward, Lushoto district.

Medicinal plants that were affected through bad harvesting methods for trade purposes were documented. Direct observation was also used to see other factors that increase the threat to medicinal plants. Important morphological characteristics that can assist in plant identification were considered during collection of plant voucher specimens and photographs were also taken. Leaves sample were also conserved in silica gel to assist in molecular identification. The specimens were subsequently deposited at the Institute of Traditional Medicine of MUHAS. Literatures used in plant identification included Nahashon (2013) Dharani *et al.*, (2010); NAFORMA, 2010; Ruffo *et al.,* (2002) and Mbuya *et al.,* (1994).

2.3 Molecular identification of medicinal plants

Fifty eight (58) powdered market samples were selected out of 306 samples for DNA barcoding based on the following criteria

1. Resembles in natural colours but having different vernacular names (19%)
2. Resembles in texture but having different vernacular names (2%).
3. Lack of scientific names- scientific names of some plants were not found both in the field and in the literatures.

Permission to transport the samples to Uppsala University for molecular identification was obtained from the Ministries of Agriculture and Natural Resources and Tourism, Phytosanitary Unit in Dar-es-Salaam.

DNA Extraction- The powdered samples mixed with silica beads to assist in grinding was transferred to the grinding machine until it was homogenized. Molecular identification of

medicinal plants was done using CTAB (Carlson/Yoon) extraction protocol (Sun *et al.*, 2013; Kool *et al.*, 2012) at Uppsala University.

The chemicals used were; CTAB buffer, mercapto-ethanol, chloroform/isoamylalcohol, NH_4 acetate, 95% EtOH and 70% EtOH. Extraction procedure involved: rough extraction, remove of proteins, DNA precipitation, and extra purification through precipitation in high salt concentration.

PCR product preparation-An initial experiment with matK barcoding primers, MatK_3F and MatK_MALPR1, yielded only one positive band for 14 samples, and after some additional experiment with annealing temperatures this marker was abandoned instead the study focused on nrITS region. The mix (25µl) for PCR contained: water (11.75 µl), reaction buffer (2.5 µl), $MgCl_2$ (2.5), dNTP (2.0 µl), Taq polymerase (0.125 µl), BSA (0.125 µl), ITS1_17SE_forwad primer (5'-ATGGTCCGGTGAAGTGTTC) (2.5µl), ITS2_26SE_reverse primer (5'-CCCGGTTCGCTCGCCGTTAC) (2.5 µl) and DNA template (1 µl).

DNA amplification -PCR product were amplified with the following thermo cycling program: At 97°C for 5 min-pre-denaturation of the nucleic acids, (97°C for 30 sec- nucleic acid denaturing and making single stranded DNA, 55°C for 1 min- primer annealing and initiating DNA synthesis, 72°C for 1 min- DNA polymerase uses the deox-nucleoside triphosphates to synthesis in 5′ to 3′ direction which result in exponential DNA synthesis) x 35cycle, 72°C for 10 min- final extension to allow any remaining single stranded DNA to be fully extended and 4°C –the amplified nucleic acid are held at this temperature until analysis.

Electrophoresis- Electrophoresis gel was prepared by mixing 1.5% agarose Tris EDTA gel (50ml) and gelred nucleic acid stains (3.5 µl) and then poured in casting tray to solidify for 20 minutes. The tray was then placed in the buffer filled box. PCR product was mixed with loading dye and filled in the gel. The electrical field was applied via the power supply to the rear. The negative terminal was at the far end, so the DNA migrated towards the anode. DNA

fragments moved in different length in the gel according to their mass and the smaller fragments moved faster than the large fragments. The gel was visualizing with UV light and gel imaging device. The image was recorded with a computer operated camera and intensity of the bands was measured and compared against ladder (Figure 1).

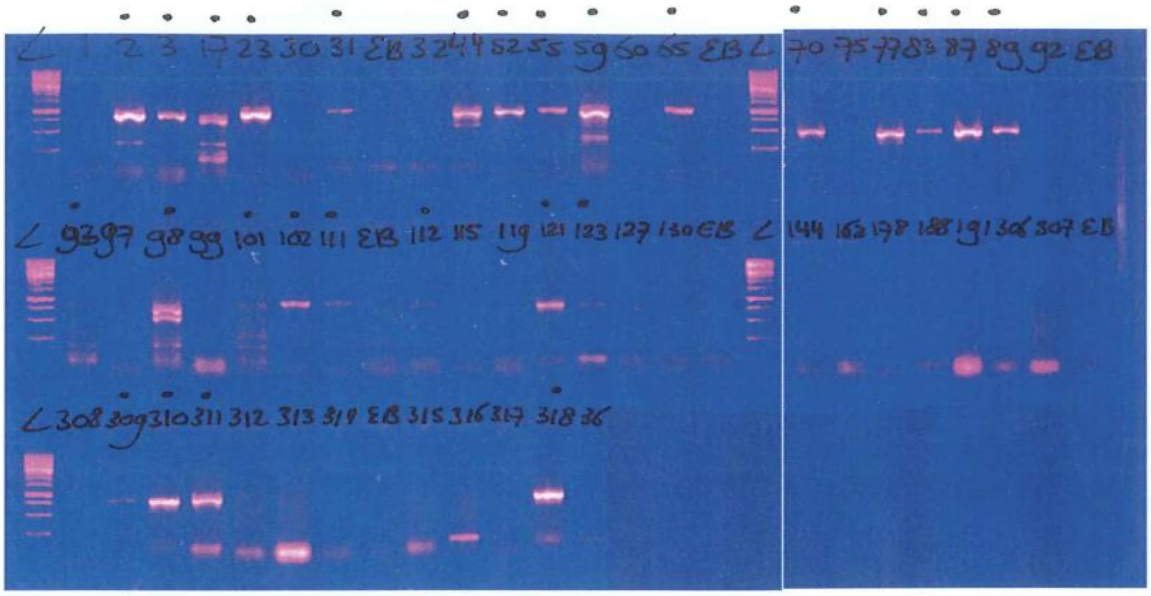


Figure 1: The bands of amplified medicinal plants DNA

2.4 Data Analysis

Data obtained from the market surveys were analysed using Microsoft Excel where percentages and frequencies were calculated. Tables and figures were drawn to assist in data interpretation.

DNA Purification, sequencing and sequences analysis- PCR products were purified and sent for sequencing at MACROGEN Company in Amsterdam, the Netherlands. The analysis of sequences was done by comparing the sequences obtained with standard sequences at Gene bank through BLAST system. The reference sequence(s) with the highest similarity score were usually selected along with maximum length of the match (E-value). All the sequence with ident score above 90% were selected as it was the case with de Boer (2014)

CHAPTER THREE

3.0. RESULTS

3.1. Traded medicinal plants in the cities

3.1.1. Characteristics of respondents

The majority of respondents who participated in the Dar-es-Salaam survey were from Kariakoo market complex (73%) where there is a special area for traditional medicine vendors, a feature not found in other markets in Dar-es-Salaam. In Tanga region, 75% of vendors were from Tanga city and 25% from Muheza town. Most (97%) of the vendors interviewed in this study were young males aged between 16 and 35 years, comprising almost two thirds of the respondents, followed by those aged from 36 years to 49 who formed just over a fifth of the respondents.

Regarding ethnicity, the majority of vendors interviewed in Dar-es-Salaam were Kwere (56%) who come from Coast region adjacent to Dar es Salaam region, 11% were from Zaramo tribe in Dar-es-Salaam and the Masaai comprised 9%. In Tanga region, the Sambaa accounted for almost two thirds of the medicine sellers while just over a fifth was Maasai. Over two thirds of the vendors had completed primary school level of education and those with secondary (ordinary) level were only a fifth. Only a few of the vendors had Islamic/Quran education and others never attended any school. Almost three quarters (74%) of the vendors both collect and sell the medicinal plants themselves. The market vendors operated from shops either with (38%) or without (47%) windows in the healing house *(Kilinge*) and 15% were found along the streets with either tables or sacks (Table 2).

Table 2: Socio-demographic characteristics of vendors on medicinal plant vendors in Dar-es-Salaam and Tanga regions

Characteristic	Frequency	Percentage
Age		
16-35	49	63
36-49	17	23
50-80	12	15
Total	**78**	
Sex		
Male	76	97
Female	2	3
Total	**78**	
Education Level of vendors		
Primary Level	54	69
Ordinary Level	16	21
None	6	8
Islamic education	1	1
Advanced Level	1	1
Total	**78**	
Religion		
Muslim	65	83
Christian	13	17
Total	**78**	
Occupation		
Seller and collector	58	74
Seller	20	26
Total	**78**	
Type of Shop		
Wooden shop with no windows(under the shed area)	40	51
Blocked meaning shop	31	40
Table on the side of the road	7	9
Total	**78**	

3.1.2. Knowledge on practices

In general, the traditional medicine vendors have extensive experience in the trade and 73% (n = 78) have been trading and practicing traditional medicine for more than 5 years. Most of traditional healers that practice traditional medicine at their home in Dar-es-Salaam visit Kariakoo market to get the medicinal plants they want. The vendors at Kariakoo were proud to have much knowledge than the healers. Several vendors from other markets such as Buguruni and Tandika, as well mentioned Kariakoo as the place where they buy the medicinal plants. A few of the vendors however, especially those from Buguruni were not able to mention the diseases that can be treated using the plants they sold. They also had experience in the market for less than 5 years.

Most vendors 80% (n=78) inherited the healing practices and the business of medicinal plants from their relatives. Among those who studied about medicinal plants, 43% (n = 10) attributed it to acquiring from ancestral spirits. Two of the traditional healers revealed that they were attending school when they were possessed by the spirits of their ancestors. Another healer was a school teacher who also claimed to have been possessed by the spirits of his forefather. Two of the healers could not mention the medicinal plants they were using, because they alleged it is the spirit which shows them the plants. One of the vendors and a healer who owes the knowledge to a spirit, refused to reveal the information for fear of offending the spirit.

Another vendor of medicinal plants refused to participate in the study, arguing that he could not do so because he was the only one chosen and entrusted and no member of the family had that right. Two of the vendors got the knowledge about medicinal plants after being cured chronic diseases with medicinal plants when they were young.

More than a quarter (27%) of the vendors interviewed said the medicinal plants have been used to cure diseases that had failed to be treated by modern medicine. A quarter (25%) of the vendors claims that modern medicines (pharmaceutics) contain chemicals that result in side effects. About a fifth (21%) of the vendors (21%) claimed that some people use medicinal plants because they are connected to them through cultures since they were young. Also

traditional medicine is said to be cheaper and accessible compared to modern medicine. In addition it is asserted when people use medicinal plants for treatment the relief is prompt.

3.1.3. Chain of medicinal plants trade

(Collector →middleman →vendor → customers)

Before the collectors harvest the medicinal plants, they have to get the permit from the village chairman. Otherwise, the collector has to harvest first and then pay later to the village chairman or inspectors on their way back based on the amount harvested. After harvesting, they grind the parts, whether leaves, roots or barks using costly machines. Sometimes they take the responsibility of transporting the medicinal plants to the city. The amount they are paid for all the process is too little compared to the big work they do.

Middlemen have to get the permit from village chairman for transporting the medicinal plants. In case he doesn't have the permit, he will face natural resource officers on their way to town; though it is very rare for them to be caught by natural resource officers. One middleman said he uses the knowledge of traditional medicine to blind natural resource officers at the check points. They supply the medicinal plants to vendors and traditional healers in town and cities.

Most vendors are also traditional healers; they have knowledge about the medicinal plants and what they treat. They buy the medicinal plants form middlemen that visit the city. They have to inform the middlemen about the species they want few weeks before their stock finish. Sometimes they take the responsibility of a middleman by traveling to the village and buy from the harvesters for their own use in the business. When they get the medicinal plants direct from the village, they take large amount than when they buy from the middlemen that visit the city.

3.1.4. Collection and harvesting methods

Overwhelmingly (92%) vendors had a lot, on average 300kg of medicinal plants in store. Only a few (8%) of the vendors failed to approximate the amount they had in store (Figure 3). Generally, a vendor can take as much as 402kg of medicinal plants per trip. Some vendors said

it depended on the plant species while others said the amount was dictated by the amount needed. A few vendors do not keep records of the amounts they collect or buy every time because it varies on each occasion.

Medicinal plants are either collected by the vendors themselves or bought from collectors and middlemen. Approximately 50 different medicinal plants were sold by one vendor. 37% of the vendors buys the medicinal plants from middlemen or collect themselves. A third of vendors collected the medicinal plants by themselves. About a fifth (19%) of the vendors only buy from collectors and middlemen that visit the market area (Table 3).

Table 3: People involved in the collection of medicinal plants

Characteristic	Frequency	Percentage
I collect by myself and buy from middlemen	29	37.18
I collect by my self	25	32.05
I buy from collectors/ middlemen that visit this market	15	19.23
I buy from collectors that visit this city and in their home villages	5	6.41
I buy from middle men here in the city	3	3.85
I buy directly from collectors in their home villages/regions	1	1.28
TOTAL	**78**	

The collection of medicinal plants is laden with a number of challenges (Table 4). Almost a third (30%) of respondents complained about the long distance from where the medicinal plants are collected. Twenty seven percent pointed out that some of the medicinal plants can only be found in specific areas within a region while some medicinal plants are seasonal. Other challenges faced during medicinal plants collection include charges from Ministry of Natural Resource and Tourism (*Maliasili*), village authorities and at the check points along the roads. It was a common complaint that permits from the Ministry of Natural Resource and

Tourism was perceived to take too long. Also collecting from most natural forests is prohibited. Occasionally the collectors encounter dangerous animals like lions and snakes. Processing and transporting the medicinal plants is also one of the problems collectors faced.

Table 4: Challenges faced during medicinal plants collection

Characteristics	**Frequency**	**Percentage**
Distance	28	30.11
Absolute availability	25	26.88
Seasonal/temporal Availability	16	17.20
Binding legislation	11	11.83
Danger animals	7	7.527
Transportation	5	5.38
Expensive to process into powder	1	1.08
Total	**93**	

If the medicinal plants are bought from middlemen, the following challenges were mentioned. 42% of the respondents stated that buying medicinal plants was too expensive while over a third (37%) complained that the middlemen did not bring the medicinal plants on time. One vendor complained that admixture can also be seen in the medicinal plant materials brought by middlemen. And also a middlemen can bring close related species that have similar features to the one needed by the vendor. Also sometimes medicinal plants are said to have norms (*masharti*) that the vendor is not aware of. Failures to follow those norms are believed to affect the efficacy of the medicinal plant.

The most well-known regions for supplying medicinal plants in Dar-es-Slaam city are Coast (24%), Tanga (18%), Tabora (15%), and Morogoro (7%) (Table 5). In Tanga city, 86% of the

medicinal plants is collected within the region and 14% originated from Arusha region. The most famous district for supplying medicinal plant to Tanga city was Lushoto which provides 60% followed by Handeni (20%) districts.

Table 5: Famous regions for supplying medicinal plants in Dar-es-Salaam city

Region	Frequency
Coast	56
Tanga	43
Tabora	34
Morogoro	18
Arusha	13
Kigoma	10
Dodoma	9

All of the areas mentioned require permits to collect the medicinal plants for trade purposes. The permits can be obtained from either the village chairman or from the Ministry of Natural Resource and Tourism. Some vendors said the ministry does not follow up what is happening in the forests.

3.1.5. Market (national and international)

A small amount (10%) of medicinal plants was reported to be exported international. Most (90%) of the time, a small amount is bought by individual patients or relatives of the patients who visit Tanzania. A few (10%) of the vendors travel out of the country to sell the medicinal plants or send medicinal plants by buses. Vendors reported that, foreign customers of medicinal plants come from Kenya (18%), Uganda (14%) and South Africa (13%) (Table 6).

Table 6: Country that export Tanzanian medicinal plants as it was reported by vendors

Country	Frequency	Percentage
Kenya	28	17.5
Uganda	23	14.4
South Africa	20	12.5
Arab	17	10.6
Zambia	14	8.75
DRC Congo	10	6.25
Burundi	7	4.37
Mozambique	7	4.37
Malawi	6	3.75
Rwanda	6	3.75
England	5	3.13

Most (94%) of the medicinal plant exported are for treating physical ailments and not spiritual like misfortune, acceptance and prosperity (*bahati*, *mvuto* and *mafanikio)*. Based on the fact that 10% (n= 78) of vendors exporting medicinal plants, the following are the medicinal plants exported: *Zanthoxylum chalybeum* Engl. (14%), *Bauhinia thonningii* Schum.(9%),*Cassia abbreviata* Oliv *(8%)* and *Zanha africana* (Radlk.) Exell (5%). (Table 7). *Zanthoxylum chalybeum* was highly traded because it acts as a preservative agent for other medicinal plants and also owing to it medicinal property.

Table 7: The medicinal plants mostly exported

Plant local name	Scientific names	Percentage
Mjafari	*Zanthoxylum chalybeum* Engl.	13.95
Kizabuni	Unidentified	8.53
Mkundekunde	*Cassia abbreviata* Oliv.	7.75
Mdaula	*Zanha africana* (Radlk.) Exell.	4.65
Kasela	Unidentified	3.88
Mpingi	*Ximenia aegyptiaca* L.	3.88
Mwifu	*Warburgia elongata* Verdc.	3.10

The commonly treated diseases to Tanzanian customers that live in Dar-es-Salaam are impotence (9%), hernia (9%), malaria (7%) and stroke (7%). The most common diseases that Tanzanians living in Tanga city seek to heal with traditional medicine are colic (11%), malaria (11%) and food allergies (9%) (n=12). The most used medicinal plants to treat those diseases are *Zanthoxylum chalybeum* Engl. (13%), *Zanha africana* (Radlk.) Exell. (5%), *Hymenaea verrucosa* Gaertn. *Cassia abbreviata* Oliv. and *Bonamia mossambicensis (*Klotzsch*)* Hallierf.

Over a third (36%) of the vendors reported that, medicinal plants are preferred because they are very effective in treating diseases and work best within a short period of time with no side effects. Many diseases that are common in Tanga were claimed by about a third (29%) of the vendors to be treated with these medicinal plants. They are also alleged to be capable in resolving psychospiritual problems such as the quest for prosperity, love charms, and impotence.

3.1.6. Parts used and preparations of medicinal plants

Most of the roots were sold among the rareness plants (Figure 2). The most commonly used part of medicinal plants was the root according to about two thirds (64%) of the vendors interviewed. This was followed by stem barks as mentioned by 17% and leaves by12% of vendors. These plants (95%) were mostly sold in powdered form and most were sold as single species. Vendors claimed to mix different species based on the patient's diseases when they

consulted the vendors for treatments. The forms in which they were sold made it hard for the medicinal plants to be identified by conventional methods and hence molecular identification was used. None plant materials were also used for treatment, including animals' parts like horns, tails and snail shells as it was observed at the markets.

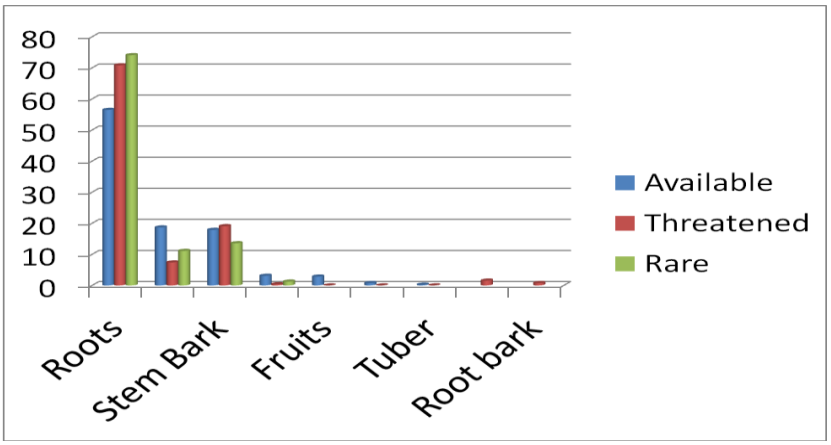


Figure 2: Parts of medicinal plants used among available, threatened and rare plants

In Tanga city, the medicinal plants were prepared by vendors who are also traditional healers. The plants were mostly prepared through infusion, followed by decoction. The medicinal plants that were used for malaria were claimed by patients to be very bitter in taste. Vendors of medicinal plants in Tanga had lists of all problems they claim to be treating; glued in a paper on the walls inside the healing site (*Kilinge*). They also post information outside the Kilinge showing that there is a traditional healer (*mganga*) inside. The people who suffered from a particular disease and benefited from traditional drugs which cured the disease become good ambassadors to others on the effectiveness of the medicine.

3.1.7. Diseases treated by medicinal plants

Medicinal plants have been used to treat different conditions. The majority (90%) of conditions mentioned by vendors concerned the body (physical) with only 10% were related with psycho-spiritual conditions. Among the physical related diseases, the following were regularly mentioned; stomach-ache (9%), impotency (8%), colic (7%) and malaria (5%) (Table 8 and Appendix 6).

Table 8: Common diseases conditions

Diseases conditions	Frequency
Stomachache	64
Importance	54
Colic	48
Heria/	38
Female Reproductive System	32
Malaria	30
Cough	19
Ulcers	18
Diabetes	17

Among the psycho-spiritual condition that were mostly consulted by patients to the vendors and traditional healers were love-charms to keep a wife/husband or girlfriend/boyfriend (30%), plant materials which bring luck in finding employment and other fortune (24%), attracting customers in the business area (10%) and those which guard against jealousy and evil spirits, to get a lover who is away (*kumvuta mpenzi aliye mbali*), hurting another person (*kudhuru*), (*kusafisha nyota)* and for prayer purposes in some beliefs.

3.1.8. Sources of income for vendors

A good number (55%) of the vendors interviewed depend on selling medicinal plants as their only means of income (Table 9). On average a vendor of medicinal plants earn 100,000 per month from his selling. The rest had other jobs that compliment their earnings. About a third (30%) engaged on other preoccupations like selling ornaments; ear rings, bangles and necklaces and one-fifth were car/bus drivers. Almost two thirds (61%) of the vendors had families of their own, and (39%) of the vendors' spouses and children had jobs that contributed to the vendors' earnings.

Table 9: Employment status of vendors

Employments	Percentage
Selling medicinal plants only	54.55
Selling medicinal plant and another job	45.455
Total	

Over half (52%) of the responding vendors said they would not mind quitting the medicinal plants trade if they had better paying jobs. About 48% of them were comfortable with selling medicinal plants. Vendors acquired the knowledge of medicinal plants through possession of spirits said, they cannot leave the trade because their ancestor spirits cannot let them quit so it was not out of conformability or loving the trade but obligation to ancestors and spirits.

3.2. Market implications to the diversity of selected medicinal plants

3.2.1. Condition for trade

The majority of vendors (59%) believed that the medicinal plants that are important in the market are at low risk of being endangered by the trade. The following are the reason they gave; the medicinal plants are many and they can be found in many regions. In case they get finished in one region they will shift to other regions. Laws of the country protect the medicinal plants, some of the medicinal plants are cultivated on farms and gardens and only small amount of the medicinal plants are harvested from the mature plants.

Over two thirds (41%) of the vendors who said the medicinal plants that are important in the market are at risk of being endangered by the trade gave the following reason: the medicinal plants will be locally extinct from current sources and will have to get them from far regions. Close to two thirds (60%) of the vendors said there are no medicinal plants that are extinct from their supplying sources. 40% of the vendors mentioned the following medicinal plants to be locally extinct in some of the supplying sources for example; *Zanha africana* (Radlk.) Exell

(20%) for 3yrs, *Synaptolepis alternifolia* Oliv. (12%) for 3yrs, *Ficus sycomorus* L. for 2yrs) and *Zanthoxylum chalybeum* Engl.for 1yr. (Table 10).

Table 10: Medicinal plants that are perceived by vendors to be locally extinct in some supplying sources

Scientific names	Venacular name	Frequency	Period (Yr)
Zanha africana (Radlk.) Exell	Mdaula	9	3
Synaptolepis alternifolia Oliv.	Msigi	5	3
Ficus sycomorus L.	Mkunju	3	2
Zanthoxylum chalybeum Engl.	Mjafari	3	1
Zanthoxylum acanthopodium DC.	Mnungununungu	2	2
Unidentified	Mangamia	2	8
Commiphora swynnertonii A. Burtt	Ndemwai	1	Many yrs
Acacia xanthophloea Benth.	Mwita	1	2
Warburgia elongata Verdc.	Mwifu	1	1
Spirostachys africana Sond.	Mwalaka	1	
Unidentified	Mvuti	1	
Strychnos spinosa Lam.	Mtonga	1	6month
Piliostigma malabaricum (Roxb.) Benth.	Mshegheshe	1	4.5
Unidentified	Msandali	1	Many yrs
Unidentified	Mrangamia	1	Many yrs
Allophylus rubifolius (Hochst. ex A.Rich.) Engl.	Mmelemele	1	1
Galiniera saxifraga	Mmavimavi	1	Many yrs
Unidentified	Mkunganilwa	1	2
Cassia abbreviata Oliv.	Mkundekunde	1	2
Unidentified	Liwa	1	
Pterocarpus mildbraedii Harms	Kulo	1	1
Unidentified	Kato	1	1
Afzelia quanzensis Welw.	Mkongo	1	Many yrs
Unidentified	Nyambu	1	
Unidentified	Kiefu	1	3

Among those who said medicinal plants were not available in the supplying sources explained that there is high demand and frequency of supply in the market. A fifth of the vendors reported that medicinal plants have other uses like timber and charcoal and that encroachment to the forest for settlement and agriculture purposes has high impacts on the existence of the medicinal plants (Table 11).

Table 11: Other uses and threat to medicinal plants

Threat to medicinal plants	**Percentage**
High demand of medicinal plants	32.26
Non medicinal uses like timber andcharcal	19.55
Urbanisation	9.68
Unsustainable harvest eg uprooting	9.68
Environmental changes	9.68
Seasonal(dry seasons)	6.45
Deforestration(burning forest)	6.45
I dnt know	3.22
Dont follow laws	3.23
No	3.23

Just over half (52%) of the vendors claimed to follow the forest laws including; harvesting the amount that can support the survival and growth of the plants which are one root per tree, vertical debarking on one side, harvesting few leaves and not harvesting young plants (Table 12). The Ministry of Natural Resources and Tourism is trying to sensitize collectors on good harvesting methods. A quarter (25%) of the vendors said they were attempting to cultivate medicinal plants like Aloe species and *Moringa oleifera* on their farms. Some associations of traditional healers like UMAWATI have farms but they don't have resources to develop. A few (15%) of them said they were not doing anything to ensure sustainable harvesting of medicinal plants. To other vendors (3%) the medicinal plants are plenty in case they diminish in one region they will shift to another region.

Table 12: Reason as to why medicinal plants will still be available

Availability of medicinal plant	**Percentage**
We follow the laws	52.56
We cultivation	25.64
No	16.67
I have special agent to supply the MP	2.56
Plants are many,cant be done	2.56

Little has been done to protect and conserve the medicinal plants in the study areas. Large amount of medicinal plants sold at the markets according to 94% of the vendors are collected from the wild. Little percentage (4.5%) is cultivated and 1.5% grows as weeds. Among the cultivated medicinal plants, only 3% of the rare species and large amount of available species (82%) were mentioned. Vendors claimed that the medicinal plants grown around Tanga city were less effective compared to those cultivated in Lushoto, Handeni and Muheza due to the inherent salty water and the soil in the area. Rare plants were not mentioned to be part of the weeds medicinal plants. Among the wild medicinal plants, 36% and 11% of medicinal plants were mentioned as threatened and rare plants respectively.

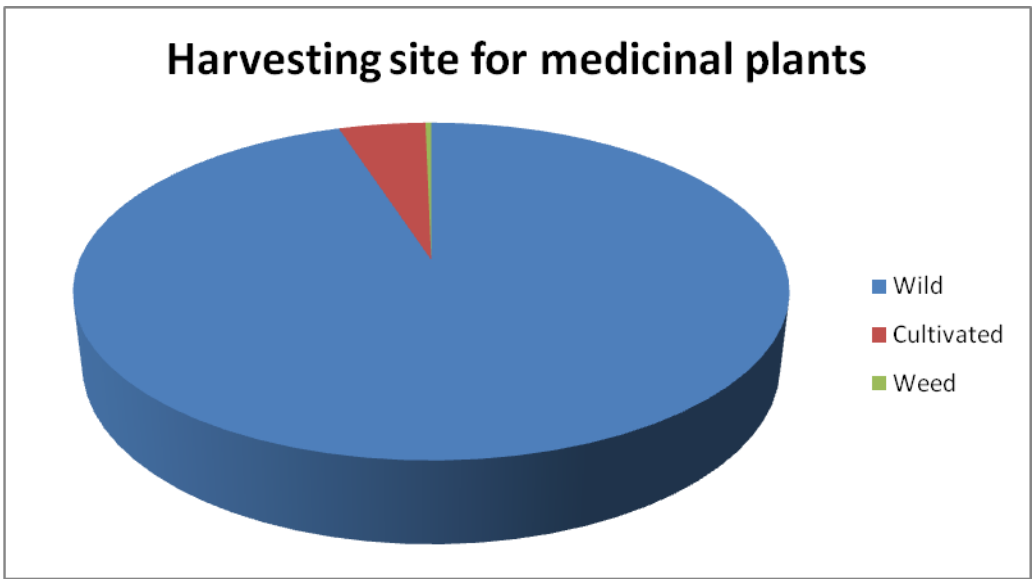


Figure 3: Harvesting sites for medicinal plant

3.2.2. Threat to medicinal plants

The most mentioned medicinal plants and considered threatened through trade (Appendix 7). Based on that criterion, the following are medicinal plants under threat: *Zanthoxylum chalybeum* Engl., Zanha *africana* (Radlk.)., Exell, *Cassia abbreviata* Oliv., *Albizia anthelmintica* Brongn., *Hymenaea verrucosa* Gaertn., *Rapanaea melanocephalus and Ximenia aegyptiaca* L.

Over half (52%) of the vendors claimed that a large number of medicinal plants were still available but about a third (35%) were of the opinion that they were threatened medicinal plants in the wild. A modest percent (10%) of the medicinal plants were claimed to be rare or not available at all. Among warning medicinal plants as claimed by collectors, the following medicinal plants were highly mentioned; *Zanthoxylum chalybeum* Engl., *Warburgia elongata* Verdc., *Bonamia* mossambicensis (Klotzsch), *Zanha africana* (Radlk.) Exell and *Cassia abbreviata* Oliv.

Based on observations in the field, some trees seemed to be highly threatened because of trade through debarking. These included; *Afzelia quanzensis* Welw. *(Mkongo*), *Hymenaea verrucosa* Gaertn. *(mkumbi*) and *Pterocarpus angolensis* (*mninga)*. Every single species of the *Afzelia quanzensis* observed in Ubenazomozi ward were entirely damaged through debarking in.

About 48% of the medicinal plants mentioned in Dar-es-Salaam were collected from Coast region. Chalinze ward in Bagamoyo district in Coast leads in providing the medicinal plants consumed in Dar-es-salaam with 97%. On the other hand the medicinal plants mentioned in Tanga were sourced mostly from Lushoto district. Above three quarter (76%) of the medicinal plants collected from Arusha region were claimed by vendors to be threatened or rare. It was followed by medicinal plants from Tanga (45%), Dar-es-Salaam (45%), Coast (40%) and Morogoro (30%) regions. (Table 13).

Table 13: Status of medicinal plants in their supplying sources as claimed by vendors in Dar-es-Salaam city

Region	Total medicinal plants	Threatened and Rare medicinal plants	Threatened and Rare %-
Arusha	66	50	76
Tanga	116	52	45
Dar-Es-Salaam	31	14	45
Coast	331	131	40
Morogoro	34	10	30

3.2.3. Status of medicinal plant during voucher specimen collection

The most mentioned medicinal plants during market survey were thought to be highly threatened. IUCN red list was used to check if those mostly mentioned medicinal plants were threatened but they were not among the red list plants.

In the villages visited in Tanga, management activities are highly compared to the villages visited in Coast regions. The Usambara Mountains where the collection took place is part of two hotspot of the world biodiversity of both flora and fauna as it was also observed during the study. The larger part of the mountain is conserved as game reserve.

Ruvu Chini in Kibaha district is full of grasslands with little diversity of plant species compared to the other villages visited in Coast. The most common medicinal plants found in Bwawa village grow as weed in a cultivated land. This shows how the collection of medicinal plants can take place in many locations, including open pasture, waste agricultural land, protected forest land and roadside. Human activities have big impact on the degradation of these forests.

Some medicinal plants were highly affected by trade through unsustainable harvesting methods. For example of medicinal plants threatened because of debarking include the following: *Albizia anthelmintica* Brongn. (*Mfuleta)* in Bwawa village and *Afzelia quanzensis* Welw.*(Mkongo*), *Hymenaea verrucosa* Gaertn.*(mkumbi*) and *Pterocarpus angolensis* Harms (*mninga)* in Ubenazomozi ward**.**

3.2.4. Road check points

The most traded plants product through Kilwa road was charcoal, timber and building poles and sometimes carvings (*vinyago).* Along Pugu road it was only charcoal transported for trade purposes. Check point officer estimated like 6-10 Lorries containing 60-70 sacks of charcoal each passing through Pugu road per day. They were not sure of which species is traded as charcoal. Medicinal plants were known to be transported but never observed by check point's officers all the time they are transported. The officers at check points were knowledgeable about the medicinal value and other uses of plants that are practiced around that region. (Appendix 7).

Among the seaports, the studies targeted are Dar-es-Salaam and Tanga. The seaport in Dar-es-Salaam reported that, they don't have the section that deals with plants product. It was

opposite in Tanga because the section dealing with plant products was there and they participated in the study. Products that are mostly transported through the Indian Ocean via Tanga seaport are timber, followed by little amount of building poles (*fito*) and animal feeds.

The species that are mostly transported as timber are naturalized *Tectona grandis* L.f. (*Tiki*) to India, followed by *Diospyros abyssinica* (Hiern) F.White (*mkuruti*) and. *Acalypha spp* (Mzundu) to China. *Leucaena leucocephala* (Lam.) de Wit (*Lusina*) is transported to Oman for animal feed. *Tectona grandis* (*Tiki*) and *Leucaena leucocephala* (*Lusina*) are harvested from plantation forest but the *Diospyros abyssinica* (Hiern) F.White (*mkuruti*) and *Mzundu* are harvested from protected forests.

The permit for harvesting and approval for exporting these products can be obtained from Tanzania Forest Services (TFS). According to the Natural resources officer of Tanga seaport, people observe the laws in this section because there is no any illegal transporter of forest product that has been captured.

3.2.5. Conservation issues

From what was observe in the field, medicinal plants in Kitonga, Bwawa and Mboga were mostly affected by human activities compared to other villages we visited. Most of the land is cleared as the human population grows. People have encroached into forest land for agricultural activities and animal grazing; trees are burnt and debarked beyond their limit to recover and end up dying. Preparation of charcoal and timber also is part of the threat to medicinal plants as it was observed in Bwawa, Mboga and Ubenazomozi villages.

3.3. Barcoding of medicinal plants

3.3.1. DNA Extraction, PCR and Sequencing

The Carlson/Yoon extraction protocol was used for all 58 DNA extractions. All the market samples were in powder dark, yellow or brown in colour except for one sample (MP162) that was green. The use of the barcoding primers for matK yielded only one PCR product out of 14 tested samples in the pilot run, and matK was subsequently abandoned. The nrITS primers yielded 10 PCR products out of 14 tested in the pilot run, and this marker was adopted for all samples. Only 27 out 58 samples (47 %) yielded PCR products for one or both of the ITS primers pairs for ITS1 or ITS2. The obtained sequences were matched to publicly available sequences in NCBI GenBank using the BLAST search algorithm.

The sequences ranging from 280-500 bp were obtained in the NCBI BLAST database. They yielded matches with species close to the putative species (based on a combination of vernacular names and morphological characteristics) in only 15% of the cases (Table14). Generally if BLAST scores of 97% were returned then these usually matched more accurately with our putative identification. Lower BLAST scores reflect a combination of poor quality queried sequences and possible absence of accurate reference species in GenBank.

Some samples (37%) medicinal plants (111, 123, 17, 23, 311, 310, 3, 98, 59 and 70) had 95% ident score and above but the highlighted BLAST result species were from different genera (Appendix 8). This made a total of 52% of samples identified through DNA barcoding. For sample MP (112, 309, 310, 318, 121, 52 and 55) the BLAST ident score were less than 90%.

Table 14: Possible species of the plants identified

Salamaki -2F_ITS17SE MP2.0.1	**E-value**	**Ident %**
Senna alexandrina	0	99
Senna alata	0	95
Senna italica	0	95
Senna didymobotrya	9.00E-180	83
Senna phyllodinea	7.00E-171	82
Mwinula- 44F_ITS17SE MP44.0.1	**E-value**	**Ident**
Ocimum basilicum	1.00E-68	94
Ocimum basilicum	2.00E-56	95
Mwinula 44R_26SE MP44.0.2		
Ocimum basilicum	7.00E-103	88
Ocimum gratissimum	9.00E-77	88
Ocimum gratissimum	4.00E-75	88
Occimum americanum	5.00E-69	88
Odongwai 77R_26SE MP77.0.2	**E-value**	**Ident**
knautia visianii	1.00E-03	100
Knautia velutina	1.00E-03	100
Knautia velebitica	1.00E-03	100
Knautia traunicensis	1.00E-03	100
Knautia subscaposa	1.00E-03	100
Kisokoza wana-87R_26SE MP87.0.2	**E-value**	**Ident**
Croton nobilis	0	96
Croton manampetsae	0	96
Croton noronhae	0	95
Croton goudotii	0	96
Croton klotzschianus	0	95

CHAPTER FOUR

4.0. DISCUSSION

4.1. Traded medicinal plants in the cities

4.1.1. Characteristic of respondents, knowledge and practices

The trade of medicinal plants is mostly (63%) done by young male below 35 years of age. This can be attributed to the scarcity of jobs in the country and little education level of these people as it was found that 80% of the vendor's hard Ordinary level of Secondary Education or Islamic level of education, or never attended school. This helps in income generation and livelihood security for most poor people in the cities (Uprety *et al.,* 2011). Few females engaged in this business compared to males because they spend much of their time taking care of the family. Female vendors were all from Masaai tribe and they were all widows. They opted to sell medicinal plants so that they can get money for their children education. They also lost their cows after they were dismissed from Mkomazi game reserve.

Vendors learned the medicinal plants and what they treat from their relatives, especially senior TMPs who are usually related. The same characteristic was reported by Tabuti *et al.,* (2002) and Kira and Komba (2012). They used to observe the medicinal plants that are offered to patients at the healing house (*kilinge*) or market area. After they are experienced and qualified, they were left to practice with the help of someone who knows the medicinal plants (Williams, 2011).

Some vendors interviewed in this research acquired the knowledge of medicinal plants through possession of ancestor's spirits. One's spirit exists before one is born and it will survive after death (Molina, 2001). Traditionally, rural African communities have relied upon the ancestors and practical skills of the medicinal plants (Cunningham, 1993; NAI, 2011). The instructions can be obtained from the ancestor's spirits while sleeping (ATPS, 2013) or through learn the medicinal plants from the forest for some years as three of the respondent in

this study reported. Two of the healers could not mention the medicinal plants they were using, because they alleged it is the spirit which shows them the plants. As pointed out by Cunningham, (1993) this make the botanical information such as ecology and scarcity of plant species revealed by spirit to be invaluable.

The vendor that refused to participate in the study disagreed that; he could not do so because he was the only one elected and delegated and no member of the family complained as it was also reported by African Technology Policy Studies (2013). This has once in a while made it to be difficult for scientists to acquire traditional knowledge for drug development.

Plants have been used since ancient times to heal and cure diseases and to improve health and wellbeing in many cultures (FAO, 2005; Marshall, 2011). Medicinal plants that are sold in Dar-es-Salaam and Tanga are symbolically significant in people's cultures and often looked as sources of power as it is supported by (Rukangira, 2001; Hamilton, 2003; Augustino *et al.,* 2011; Kira and Komba, 2012).

Majority of people in Dar-es-Salaam and Tanga cities have migrated from rural areas and are still attached to their traditions and still hold traditional values of handling diseases. More than a quarter of Vendors reported that, people use medicinal plants because they cannot be cured by modern medicine. For examples chronic diseases like cancer, HIV's erectile dysfunction, diabetes (Kira and Komba, 2012; McMillen, 2012), mental illnesses and traditional diseases (psycho-spiritual conditions) (Mwaseba *et al.,* 2000; Molina, 2001; Struthers, 2004; Gone, 2010; Williams, 2011; Mackenrodt, 2011; Augustino *et al.,* 2011) cannot be handled by pharmaceutics. Phsychospiritual problems are due to high prevalence of severe mental disorders, increase in stress-related disorders and an increase in domestic, physical, and psychological violence (Atindanbila and Thompson, 2011).

Some vendors have related the chronic diseases like cancer to witchcraft or psycho-spiritual diseases. Such diseases can only be treated traditional as it is supported by other researchers

(Atindanbila and Thompson, 2011; Living, 2011; ATPS, 2013). Vendors interviewed supposed that, pharmaceutics products have chemicals that result in side effect which is not the case with medicinal plants.

According to vendors of medicinal plants interviewed, traditional medicine is cheaper and accessible compared to modern medicine as it is supported by (Shanley, 2003; Hamilton, 2003; Rukangira, 2001**).** Treatment is offered to the patient and later after being cured he can come back and thanks the healer based on what he/she has. When people use medicinal plants for treatment the relief is fast. Western-type medical facilities have not been able to cope with the rapidly growing urban population (Cunningham, 1993), so medicinal plants is supplementing in solving the problem.

Learning the medicinal plants because of money can drive people to cheat when it comes to rare plants. The acquisition of knowledge through ancestor spirits can be accompanied by avoiding the authority powers. This is why the always refuse to reveal the names and uses of plants for fear of the spirits. Thus why, DNA barcording can be applied to reveal the medicinal plants that are claimed to be hidden by ancestor spirits. It can also be used to identify the illegal trade in medicinal plants in the market.

4.1.2. Collection and harvesting methods

Based on collectors of medicinal plants interviewed in Pwani region, most of the collection took place in the forest land and little was collected from the farm, garden or along the road side. This is in contrast with the study done in Same district, Kilimanjaro region by Abihudi (2012) who found that the plants “weeds” were found in agricultural or waste land.

Vendors (37%) collect the medicinal plants for trade or buy from middlemen and collectors. The collectors are often economically marginalized sections of the population (Hamilton, 2003). Those who buy from middlemen and collectors that visit the city lack people to run their business when they are away. Amongst vendors who collect the medicinal plants by their

own is because they are certain of the collections they make. In addition, they have people to assist them in their business when they are away. The cost of collecting and transporting the medicinal plants is almost the same as buying from collectors that visit the city. DNA barcording can be used to authenticate the quality of medicinal plants as it passes from one business person to the other.

Distance is one of the burdens the collector face during medicinal plant collection as it was also reported by Forestry and Beekeeping Division, (1999). The market price of medicinal plants was observed to be elevated if the plant is rare as it was also reported by Rashid, (2009). Some of the medicinal plants can only be found in specific areas within a village or a region. Also it is to be appreciated that some medicinal plants especially herbs can only blossom during the rainy season. During the dry season some medicinal plant are glued tightly to the soil to the point that they cannot be uprooted. Some medicinal plants such as *Salvadora persica* L *(Msiganyika)* were only capable of growing on anthill. For such plant, it will be hard to grow in ex-situ environment. The agro-climatic requirement specificity, limit the medicinal plants which are amenable for cultivation (Singh, 2006). Transportation of medicinal plants materials from harvesting areas to town was also a problem mentioned by collectors and vendors of medicinal plants in this study as it was the case with FAO, (2005).

High demand and long distance in searching of medicinal plant can tempt collectors to unfaithful collect any available plants for the sake of money, DNA barcoding be used to determine the effect of long distant and demand of medicinal plants in the market.

Large quantities of medicinal plants were traded to urban of Dar-es-Slaam and Tanga city from rural areas as it was the case with mostly developing countries (Hamilton, 2003). This work is mostly done by middlemen and collectors. Sometimes vendors take long to bring the medicinal plants they are ordered to bring. The admixture has also been a problem when buying the medicinal material from middlemen and collectors. Middlemen have confused

closed related species and bring plants they were not asked. DNA barcoding can be used to solve issue of admixtures.

The collection of medicinal plants took place in Coastal region than other areas because Coast is very close to Dar-es-Salaam compares to other regions. In addition, most of the vendors interviewed in Dar-es-Salaam come from Coastal regions. In Tanga, Lushoto was leading in providing the medicinal plants that were sold in Tanga town. This is because Tanga is rich of biological diversity and most healers came from this district (*Sambaa tribe*) (McMillen, 2008).

Collectors get the permit to collect medicinal plants from village chairman or Ministry of Natural Resource and Tourism. Some middlemen and vendors said that, ministry responsible is not strict in ensuring medicinal plants collectors have permits before collecting, this increase the exploitation of medicinal plants. The unregulated collection of these plants has the potential to endanger plant species survival in the wild (Marshall, 2011). Much of the medicinal plant traded are unrecorded in official statistics or poorly documented. This is the reasons as to why there is typically so little awareness to decision-makers of the significance of the trade to the healthcare and economies of their people (Hamilton, 2003). The knowledge of DNA barcoding can be applied by TFS in controlling the trade of threatened medicinal plants.

4.1.3. Market (local and international)

The trade in medicinal plants has been observed to be more domestic in this study, to a large extent within nearest regions as it supported by Hamilton (2003). Small amounts are exported, and for this reason there is little risk to medicinal plants due to exportation. This is conflicts with the WHO report of 2005 which pointed out that international trade on medicinal plants was high and increasing. The trade in wild plants is also very poorly documented (Uprety *et al.*, 2011; Hamilton, 2003).

In this research, some medicinal plants were harvested for other uses and among those plants, some were international exported. Those plants included *Afzelia quanzensis (mkongo)* and *Polyscias fulva (P. kikuyuensis)* for timber, *Sureganda zanzibariensis*, *Ximenia Americana* (*mpingi*), m*sakula nguku/mwale* for building poles as it was reported by Natural resource officer of Kisarawe check point. Non medicinal purposes like timber and charcoal increases the depletion of medicinal plants (Mwaseba *et al.,* 2000; MCFP, 2004; Giliba *et al.,* 2010; Schaafsma *et al.,* 2011; Sill *et al.,*, 2011).

4.1.4. Vendor's source of income

A good number (55%) of vendors and other people interviewed in this research gain their income from wild harvest or are involved in the trading or processing of medicinal plants. Those vendors that depend on selling medicinal plants as their only means of income are more liable to suffer mostly from loss of medicinal plants because of high degrees of dependency on their local natural environments (Hamilton, 2003; McMillen, 2012; Uprety *et al.,*, 2011). The rest of vendors (45%) have other jobs include selling ornaments like; hearings, bracelets, bangles and necklaces. Some vendors have family of their own, and few members are working and support on the little a vendor brings at home. This adds on their income of a vendor (Schaafsma *et al.*, 2011).

Most vendors said they will agree to leave their jobs in case they get a better paying jobs rather than selling medicinal plants. Lack of alternative livelihood activities has increased the pressure on medicinal plants (uprety *et al.*, 2011).

4.2. Market implication to the diversity of medicinal plants

4.2.1. Conditions for trade

Medicinal plants were observed to be threatened, mainly because of habitat loss and habitat degradation in Mboga village, also over-harvesting as it was the case in other studies (Motaleb, 2010; Uprety *et al.,* 2011). Medicinal plants are threatened by unsustainable harvest by uprooting and debarking parallel as it was observed in Ubenazomozi Village.

Rare and threatened medicinal plants were mentioned by vendors to be collected from the wild. The 36% and 11% of medicinal plants were mentioned as threatened and rare plants respectively. Rareness based on the vendors was the scarce of the medicinal plants in their supplying sources and their demand in the market was high.

In case the medicinal plants get finished in one region, the collectors will shift to the nearest regions. This will result into local extinction of the medicinal plants from current sources which will increase the distance from where they will be harvesting in the future (FBD, 1999). Local extinction of medicinal plants adversely affects collectors who previously gathered medicinal plants in those sites (Cunningham, 1993).

Rare plants were documented to be harvested from far regions than available plants. The species specific nature of the demand for medicinal plants is responsible for generating long distance trade across regions and international boundaries (Cunningham, 1993). Over-harvesting of naturally growing medicinal plants and shifting to other regions after they get finished discourages local cultivation of medicinal plants (Motaleb, 2010). Local, national or commercial extinction of medicinal plants should not be under-estimated (Hamilton, 2003)**.** There can be serious consequences for livelihoods and economies if no measure will be done.

Our daily activities causes environmental change which lead to long period of dry season. Extended dry season make hard to get some medicinal plants that are essential in traditional medicine healing. The laws that protect the plants are there but not followed as it was reported by some vendors and as well supported by Uprety *et al.*, (2011).

This study shows how the increase in demand of medicinal plants and their habitats was threatening many species harvested from the wild. The most vulnerable species were popular species like: *Zanthoxylum chalybeum* Engl., *Zanha africana* (Radlk.) Exell and *Cassia abbreviata* Oliv as it was the case in this study. Other vulnerable species include slow growing or slow to reproduce or species with specific habitat requirements and a limited distribution

(Cunningham, 1993). Many plant species have become extinct and some are endangered as the result of indiscriminate harvesting (Rukangira, 2001).

4.2.2. Management of medicinal plants

A large amount of the medicinal plants (94%) is collected from the wild. This agree with the research done in South Africa with 99% of the 400-550 species sold for use in traditional medicine originated from wild sources (Hamilton, 2003).

Effort to cultivate the medicinal plants is very low with only 4.7% of vendors cultivating the medicinal plants; this is insufficient to meet the growing demand of medicinal plants. Large scale cultivation of medicinal plants can only be seen in number of species that are used as starting points for pharmaceutical drugs such as *Artemisia annua* L. in Arusha region**.**

Commercial cultivation of most species is not simple due to the slow growth rates for most tree species and low prices paid for traditional medicines. These slow growing species are a priority for *ex situ* conservation and should be strict protected in core conservation areas (Cunningham, 1993; Mwaseba *et al.,* 2000; Marshall, 2011).

The medicinal plants vendors in Tanga town were complaining about little amount of active ingredients on cultivated medicinal plant. This can be influenced by the soil and water where the plants were grown as it was also reported by Marshall (2011), Bussmann and Sharon, (2009) and FAO (2005)

The medicinal plants for instance *Acacia nilotica* (*Kiloriti)* along Ruvu River, and *Cassia abbreviata* (*Mkundekunde)* in Bwawa village can be planted as part of conservation because, they have seeds. The only limitation can be environmental conditions for plant such as *Salvadora persica* L. (*Msiganyika*) in Bwawa village which was mostly found growing on the termite mount. Favorite's environmental conditions are required in order for such a plant to grow in ex-situ environment.

4.2.3. Diseases treated by medicinal plants

Roots were mostly used in treating diseases than any other part of plants followed by barks. It is hard for the plant to survive if the root is removed or disturbed (Marshall, 2011). In practice, sustainable harvest and use of roots, barks, or whole plants used as herbal medicines are impossible (Cunningham, 1993; Rukangira, 2001; FAO, 2005; McMillen, 2008; Marshall, 2011). Roots of rare or endangered species could inadvertently be collected if they are easily confused with more abundant relatives (de Boer *et al.,* 2014). This increases the scarcity for many of the medicinal wildlife species.

The medicinal plants in Dar-es-Salaam were mostly offered in powdered form as a single species. The vendor mixed different species powders only when the patients mentioned the disease. The combination of these different plants is claimed to cure several ailments. It is believed that, each active principle of component plant making up the recipe complement one another in the fight against diseases (Odugbemi *et al.*, 2007).

The medicinal plants in Tanga were mostly of root or back form. They were observed to be prepared by the vendors who are also healers in Tanga town. The medicines were mostly prepared through infusion method. Other method like decoction and tincture were also used as it was the case with Odugbemi *et al.,* (2007). The medicinal plants that were used for malaria were claimed by patient to be very bitter in taste as it is also supported by Odugbemi *et al.,* (2007).

The traditional healers in Tanga are confident with what they do. This is because they have lists of all problems they claim to cure in their walls inside the healing house (*Kilinge*). This is a means of establishing their business (Kira and Komba, 2012). They also put a post outside the healing house showing that there is a traditional healer (*mganga*) inside.

True healer does not advertise his healing power. People who get cured from their diseases become good ambassadors to others on the effectiveness of the drug. Those healers that

advertise their medicines at a very high cost through broadcasting media like televisions, radio and in news papers; it is likely that they are blackmailing the society (Kira and Komba, 2012).

Medicinal plants have been used to treat different conditions like physical (90%) and psycho-spiritual (10%) conditions. Traditional medicine takes a holistic approach: good health, disease, and success or remove misfortune are not seen as chance occurrences but are believed to arise from the actions of individuals and ancestral spirits according to the balance or imbalance between the individual and the social environment (Cunningham, 1993; WHO, 2005; Williams, 2011). Medicinal plants are also used to prevent other social problems which have never been addressed by the western science and technology like theft, divorce and witchcraft as it was observed in Tanga and supported by Kira and Komba (2012).

Some medicinal plants like *Mvuto, Kisakulanguku* and *Mnamata* re claimed to be source of attraction, both for love affairs and business issues. This finding is supported by the research done in Dar es-Salaam by Kira and Komba (2012). Some of the medicines are said to be effective to remove bad luck and to reinstate those who have been bewitched into normal conditions, to harm disliked persons and to remove evils spirits. Medicinal plants are symbolically important in many cultures, often being seen as sources of power (Hamilton, 2003).

4.2.4. Road check points

Medicinal plants are known to be transported but never observed or caught all the time they are transported. This can be explained by little amount that cannot be observed and the public transportation they use. The middlemen and vendors also use local knowledge to blind the check point's officers not to recognise medicinal plants during transportations. This make hard to estimate the amount of medicinal plants transported and illegal trade in medicinal plants.

Medicinal plants have been documented in this research to have other uses like timber, charcoal and building poles, other uses of medicinal plant create more threat to medicinal plants (Cunningham, 1993; Giliba, *et al.,* 2010; Sill *et al.,* 2011).

4.3. Medicinal plants identified by dna barcoding

The powdered samples extracted were all in brown, dark yellow and black except for one sample which was green (SA 162). This means most of the samples were collected from the bark, stem and root which is hard to extract the DNA from such parts of the plants. This agree with the study done in Tanzania by (McMillen, 2008) that, bark and roots seem to dominate the medicinal plant markets accounting for up to 50% of the plant products sold. This can be explained by longer shelf-life of roots and barks, and can be transported over longer distances without perishing. When working with such plants parts, plant identification is much more cumbersome (Van Andela *et al.,* 2012). This is because the CTAB extraction protocol was designed for soft leaves.

The more the fresh (Janzen, 2009) and the green the sample is, the more the chlorophyll will be and simpler the extraction will work out well (Janzen, 2009). This can explain why some samples (53%, n = 57) didn't show the PCR product after gel electrophoresis. Therefore, DNA barcoding can be successful based on the plant part used.

The matK barcode was successful for only one sample hence decided to change to ITS barcode as it was the same case with Kool *et al.,* (2012) and Bafeel *et al.,* (2011). ITS is more broad in plant identification than the matK barcode (Sun *et al.,* 2013). MatK barcode deals with plastid only while the ITS deals with nuclear. ITS is the barcode, found in both plants and fungus. The ITS region can give the ideas of what species, genus, family or class the plant is.

The standard PCR protocol was designed for young and soft tissues. The samples extracted were from root and barks which are hard and contained a lot of impurities. For the samples that didn't show PCR products in the electrophoresis gel, it might be due to insufficient temperature or time. The PCR temperature is moreover important in determining how successful the primers will work out.

Identified sequences of some medicinal plants did not resemble any plants from African when PROTA database was used to check their origin. The achievement of any method used to assign sequences to a certain taxon is eventually reliant on the taxonomic coverage of the reference database (de Boer *et al.*, 2014). For some (15%), their closely related species were found and they were identified up to genera level as it was the same with Linder *et al.*, (2000).

For others species (37%) were not supported by any literature but their close relative genera were found with the BLAST ident score above 90% as it was also a case with de Boer *et al.*, (2014). Among those which did not resemble the sample identified conventional, the conventional method might be wrong as it was the case with Stech *et al.*, (2013). It can be also due to contamination of the samples due to mix-up at the market without later checking the material microscopically (Stech *et al.*, 2013), or it may be one vernacular name of the medicinal plants is used by different tribes and it means different plants or more vernacular names for one species, but might also be an inherent problem of the diagnostic characters (Stech *et al.*, 2013). In a research done by de Boer *et al.*, (2014) barcoding identified the substitute sharing the same chemical properties to the plants documented on the literature were identified.

4.3.1. Information that support the species identified through DNA barcording

The scientific names obtained through BLAST (MP 44) resembled the information documented after the conventional identification of medicinal plants was done as it was a case with de Boer *et al.*, (2014) and Gu *et al.*, (2013) in their samples. The following are the possible species identified through DNA barcoding as it was shown in Table 14.

MP 2- Salamaki

Based on the botanical information collected from this study, this species was sold at Kariakoo market only. It was mainly used for stomach cleansing and for reducing weight. The websites supports it to be Senna species without the exactly scientific name. Based on the sequences

result obtained from NCBI BLAST database, the species can either be *Senna alexandrina* (99%), *Senna alata* (95%) or *Senna italic* (95%).

The species have the same characteristic with the *Senna alexandrina* of laxative for constipation and for reducing weight (Schmelzer and Fakim, 2008). With this support and highest ident score of 99% the plant is identified as *Senna alexandrina.* *Senna alata* and *Senna italic* had the same effect and is found in Africa, the former is mainly used for skin problems urine problems, kidney stone, joint swelling and for cleaning blood (Mohideen *et al.*, 2005). The later is also used for fever, jaundice, venereal diseases and and have abortive effect (Ahangarpour and Oroojan, 2010).

The name salamaki is also used as trade name for Indian senna products and senamekki for Arabs senna. It might be possible that, the Tanzanian traders have adopted the name from Indians after they found out the plant have same effect as the one from India. Base on two vendors, this product is mostly preferred by Indians and Arabs that are living in Dar-es-Salaam city. It might also be that, the product is from India and not originating from Tanzania. The distinction made in trade between India Senna and *Alexandrian senna* from Sudan has no taxonomic basis (Schmelzer and Fakim, 2008).

MP 77- Mwinula

The reference to herbarium supports it to be *Ocimum americanum*, but with the highest score and repeated many times by forward and reverse primers identify it as *Ocimum basilicum.* The uses of plants were mentioned by vendors to be diseases (pressure and importance) and non diseases condition (prosperity, love affairs and for mediation purposes). It uses differs with what have been published by other scientist has been recommended for the treatment of headaches, coughs, infections of upper respiratory tract, kidney malfunction and to eliminate toxins (Unnithan *et al.*, 2013).

MP 77- Ondongwai

The medicinal plant was morphological identified as Commiphora swynnertonii A. Burtt (Burseraceae). The DNA barcording resulted to *Knautia spp* with *K. velutina, K. velebitica, K. traunicensis* and *K subscaposa* (Caprifoliaceae), all with100 ident score. These species have the same characteristic of treating wounds, sores and scabies. The powdered plant sample might be mixed up with other things that lead into wrong identification.

MP 87- Kisokoza wana

The plant was identified by DNA barcoding to belong to *Croton spp with C. nobilis* (96%), *C manampetsae* (96%), *C. goudotii* (96%), *C. noronhae* (95%) and *C. klotzschianus* (95%). The species can be either *C. goudotii*, or *C. noronhae* because they were the only species that can be found in Africa. Ethno botanical information collected during the study explains the plant to be used for headache, migraine and epilepsy. Other study by Volken (1999) explain the species to be used as an abortifacient and Ofusori *et al.*, (2007) said it increases sperm concentration and sperm motility.

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

There is great threat in medicinal plants, mostly on important species in trade. The nearest regions and homelands of most vendors are the one supplying medicinal plants to Tanga and Dar-es-Saalaam cities. The trade in medicinal plants has affected the availability of medicinal plants in those regions. Little has been done to conserve these medicinal plant *in-situ* and *ex-situ*. Molecular identification of medicinal plant samples sequenced was successfull by 52% to atleast to genus level. Forexample *Ocimum americanum* sample was successfully identified to the species level. DNA barcoding in this case indicated that some medicinal samples in the market are traded using wrong names. This method can be applied to solve identification of medicinal plants that are in forms which can't be identified through conventional methods. Some samples (37%) identified through conventional methods were proved to be something else through DNA barcoding.

5.2. Recommendations

Ministry of natural resources and tourism and other institutional responsible for medicinal plants should increase awareness on plants conservation and sustainable harvesting by management of natural forest, research and training in conservation both in-situ and ex-situ. Laws that are responsible for conservation of medicinal plants should be accentuated and implemented.

Ministry of energy must promote other alternative sources of energy to reduce consumption of fire wood, and charcoal as the NEAP (National Environmental Action Plan) emphasizes.

There should be an inventory of the species in the wild to know it status and prove or disprove the existing claim of threat to medicinal plants by medicinal plants vendors. More emphasize

should be put to those region like Coast and Arusha with high threat and to the medicinal plants that are jeopardized due to trade**.**

Vendors who have not started cultivating medicinal plants should be encouraged through their association to have botanical garden in their yard or field. This will help in conserving and reduce the distance from collection sites.

Some associations of vendors have plans to cultivate medicinal plants but they don't have enough resources to start. Their willingness can be supported by conservation programs, government and NGOs. Effective collaboration, communication and coordination between all these stakeholders concerning conservation of medicinal plants are required for securing sustainable development.

More fund and time should be available to accomplish the leaves sample not identified so that, their sequences can be compared with the powdered sample which lacked scientific names.

The standard protocol for extraction and PCR should be flexible to fit different part of the plants especial those in forms like powdered, roots and barks which are difficult to extract for DNA amplification. Universal primers like ITS can be used to give an ideas of which class, family or genus the plant is before other specific primers can be designed.

After the 52% success of molecular identification, DNA barcoding methods can now be applied to solve problem of wrong identification of criptic forms of medicinal plant samples (semi processed and processed) that can't be solved through conventional method. This method can be apllied in controlling of illegal trade of endangered medicinal plants.

Tanzania is lacking material and expertises to run DNA barcoding. More laboratories should be constructed and all necessary equipment should be bought to solve the wrong identification of plant. People in departments related to plants should be taught this new knowledge to assist in identification through molecular method.

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APPENDICES

Appendix 1: Consent form: English version

STUDY TITLE: DOCUMENTATION AND IDENTIFICATION OF MEDICINAL PLANTS THREATENED BY TRADE IN TANZANIA BY MEANS OF MOLECULAR DNA BARCODING

My name is Siri Abihudi, a 2nd year student doing Masters Degree at theTraditional Medicine Development Institute of Muhimbili University of Health and Allied Sciences (MUHAS).

I am conducting a study with the above title as part of my study program.

Aims of the study

This study aims to document and identify medicinal plants threatened by trade in Tanzania by means of molecular DNA barcoding. This will help in coming up with better conservation method of potential Medicinal plants.

Participation in this study:

During this study, you are requestedto respond to some questions about Medicinal plants and their trade. This interview is designed to take approximately a half 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 need of conserving medicinal plants will increase to the government of Tanzania and traders. This will be by coming up with feasible

conservation strategies of both in-situ and ex-situ. You will have contributed in rectifying any problems arising from the trade in medicinal plants.

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
   ITM
   MUHAS
   P. O. Box 65001,
   Dar es Salaam.
   Cell no: +255 712 958 211
   Email: sabihudi@gmail.com
2. The supervisors,
   Dr. Nicolao Joseph Otieno
   Cell no: +255 784 412 625
   Dr. Hugo Joris de Boer
   ITM
   MUHAS
   P. O. Box 65001
   Dar es Salaam

3. IRB chairman,

Prof. Mainen Moshi

P. O. Box 665001

MUHAS

Simu no: 2150302-6

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 2: Consent form: Swahili version

FOMU YA RIDHAA YA KUSHIRIKI KATIKA UTAFITI

Jina langu ni SIRI Abihudi, mwanafunzi wa mwaka wa pili katika fani ya tiba asilia katika Chuo Kikuu cha Afya na Sayansi Shirikishi: Muhimbili (MUHAS). Nafanya utafiti kuhusiana na hatari ipatayo Miti dawa kutokana na biashara katika masoko ya Dar-es-Salaam na Tanga.

Madhumuni ya utafiti: Utafiti huu unalenga kunakili na kutambua Miti dawa iliyo hatarishwa na biashara kwa njia ya DNA barcoding.

Ushirika katika utafiti huu: Utafiti huo hasa unahusu kujibu maswali kuhusu hatari ziipatayo miti dawa kutokana na biashara. Mahojiano haya yanatarajiwa kuchukua pungufu ya nusu saa. Jisikie huru kuongea kuhusiana na hoja hii na mengine mengi yanayo husiana na hoja hii. Kama kuna swali hujalielewa au hujisikii kujibu tafadhali usisite kusema tuendelee na swali jingine; au kama huta jisikia huru kuendelea na mahojiano unaweza sema pia.
Hatari: Hatutarajii kwamba hutakuwa na hatari kwa kushiriki katika utafiti huu.

Faida za utafiti: Kwa kushiriki katika utafiti huu, uelewa kuhusu utunzaji Miti dawa kwa Serikali na wauzaji utaongezeka. Hii itakuwa kwa kubuni mbinu mpya ya kuhifadhi Miti dawa mahali asilia upatikanapo au kwa kuotesha katika eneo jingine.

Usiri wa taarifa: Taarifa binafsi zote zitahifadhiwa mahali pa siri. Sisi pekee na timu inayo husika na utafiti huu zitaruhusiwa kuona dawa za miti hiyo. Matokeo ya utafiti huu yatawekwa kwenye mtandao.Baada ya kumalizika kwa utafiti huu,taarifa zote binafsi na miti dawa ita haribiwa au kuhifadhiwa katika sehemu salama.

Malipo:
Kwa kushiriki kwenye utafiti huu, hautalipwa wala hautalipa chochote.

Ukiwa na swali au tatizo lolote, unaweza kuwasiliana na wafuatao:

1. Mwanafunzi mtafiti,
   Siri Abihudi
   ITM
   MUHAS
   P. O. Box 65001,
   Dar es Salaam.
   Simu no: +255 712 958 211
   Email: sabihudi@gmail.com

2. Wasimamizi wa utafiti,
   Dr. Nicolao Joseph Otieno
   Simu no: +255 784 412 625
   Dr. Hugo Joris De Boer
   ITM
   MUHAS
   P. O. Box 65001
   Dar es Salaam

3. IRB chairman,
   Prof. Mainen Moshi
   P. O. Box 665001
   MUHAS
   Simu no. 2150302-6

Makubaliano ya mshirika

Mimi, _____, nimesoma/nimesomewa maelezo yote yaliyomo kwenye fomu hii na nimeelewa. Nakubali kushiriki katika utafiti huu.
Sahihi ya mshiriki _____
Sahihi ya Mtafiti _____ Tarehe _____

Appendix 3: Questionnaire English Version

Questionnaire to the vendors

a. Site data

City / Town	
Market	
Date	

b. Biodata

Name		Religion	
Age		Tribe	
Sex		Main occupation	
Education		Collector/supplier	

c. Trade and threat to Medicinal plants

1. When did you start selling Medicinal plants……
2. What encouraged you to start this business of Medicinal plants
   a. It pays a lot
   b. I inherited
   c. I studied about medicinal plants
   d. Others (explain).....
3. Why do you think people prefer Medicinal plants?.....(choose from below multiple answers possible, please rank them)

a) People want medicinal plants

b) People are not cured by modern medicine

c) There are no dispensaries close to the people

d) Medicinal plants are affordable

e) Other reasons (specify)……,.....

4. How many different medicinal plants are you selling? ……
5. How do you get the Medicinal plants (choose from below)
    a. I collect by my self
    b. I buy from collectors that visit this city.
    c. I buy from middle men in here in the city.
    d. I buy directly from collectors in their home villages/regions
6. If 5a above, then: What are the challenges you face in collecting your medicinal plants. Please elaborate/explain.
    a. Absolute availability
    b. Seasonal/temporal availability
    c. Distance
    d. Binding legislation
    e. Others challenges (specify)……..
7. What are the challenges involved in buying from middle men
    a. Costs
    b. Timely delivery
    c. Adulteration
    d. Others challenges (specify)……..
8. Which regions are famous for supplying most of medicinal plants your selling? ……
9. Do any of these areas require permits? ……. If yes, please mention these areas ……
10. a. How many times in a year do you take the stock ?……
    b. How much do you take at a time? …….
11. How much do you have in stock right now?.....
    ……

12. Do you sell you Medicinal plants abroad?.....(Choose from below)
    a. Yes

    b. No

13. If yes, do you do it yourself or are they sold by someone else? ……..
14. Which countries are the most importers of Tanzania Medicinal plants?.....
    .....
15. Please mention medicinal plant that are sold abroad ……
    …….....
16. Which medicinal plants are famous in this market? .....
    .....
17. What make them more famous/ preferred? ……
    …….
18. Do you think by being famous it affect some medicinal plants? ……how?
    ……..……
    ……..……
19. Are there Medicinal plants that are no longer available in the supplying source? Please mention them……
    for how long now? …….
20. What caused the mentioned medicinal plant to be no longer available? .....
21. What do you do to ensure the sustainable harvesting of medicinal plant
    …….
22. Do you have any other jobs in addition to selling plants?.....
    If yes, how many days/week or hours/days……
23. Do you have a family (wife/children)? ……. If yes, do your wife and children work too? ……
24. If you could get other work than selling products in a shop, would you take that job instead? ……..
25. Please list the Medicinal plants your selling and other information needed in the table below.

Plant species	Management status C=Cultivated W=Weed Wi=Wild	Part used: *Fr= Fruits* *Fl=Flowers* *L=Leaves* *SB=Stem bark* *SW=stem wood* *ST = Whole stem* *RB=Root bark* *WR=Wood root* *RT = whole root* *WP=whole plant* *Other e.g. galls*	Region	Harvest method *Uprooting* *BH-Barking horizontally* *BV-Barking vertically* *CW-Cutting The Stem* *L- Leaves* *Fruits* *Flowers*	Status *A- Abundant* *T-Threatened* *R-Rare* *E-Extinct*	Disease treated

Thanks for your participation

Appendix 4: Swahili version

Fomu ya Maswali/Mahojiano

Kwa wafanya biashara wa mimea dawa

a. Eneo

Mji	
Soko	
Tarehe	

b. Taarifa binafsi

Jina		Dini	
Umri		Kabila	
Jinsia		Kazi Mkusanyaji/muuzaji	
Elimu			
No. ya Simu			

c. Biashara ya mimea dawa

1. Ni lini ulianza biashara ya kuuza miti dawa ……
2. Je ni kitu gani kili kukuvutia/ kilikushawishi kuanza biashara hii ……(chagua chini)
    a. Inafaida kubwa
    b. Nili rithishwa
    c. Nilisomea
    d. Nyingine……

3. Unafikiri ni kwanini watu wanapenda dawa za miti shamba?.....(chagua jibu zaidi ya moja chini kulingana na ukweli wake)
    a. Watu wanataka mimea dawa
    b. Watu hawaponi na dawa za hospitalini
    c. Hakuna huduma za afya karibu na watu
    d. Mimea dawa ni bei nafuu
    e. Nyingine (dadavua)……
4. Ni mimea ya aina ngapi unayo uza?.....
5. Je unapataje miti dawa? (chagua chini)
    a. Ninakusanya mwenyewe
    b. Ninanunua kutoka kwa wauzaji wanao tembelea jiji hili
    c. Ninanunua kutoka kwa wauzaji walioko hapa mjini
    d. Ninanunua kutoka kwa wauzaji walioko katika vijiji husika
6. Ni changamoto gani unapata katika kukutafuta na kukusanya miti dawa?
    a. Upatikanaji kwa ujumla
    b. Upatikanaji wa msimu
    c. Umbali
    d. Sheria zinawafunga
    e. Nyingine (dadavua)……
7. Ni changamoto gani unazipata unapo nunua miti dawa kwa wakusanyaji na madalali?
    a. Upatikanaji wa mimea dawa kwa wakati
    b. Miti dawa inachakachuliwa
    c. Miti dawa inakua na taka nyingine
    d. Nyingine (dadavua)……
8. Ni mikoa gani ni maarufu kwa kutoa miti dawa inayo uzwa hapa ……
9. Je kunaeneo kati ya uliyo itaja inahitaji kibali?.....tafadhali orodhesha maeneo yanayo hitaji kibali ……
10. a .Ni safari ngapi kwa mwaka unachukua mzigo wa miti dawa kutoka huko unapo itoa

    ……..…….…….

    b. Ni kiasi gani unachukua kwa safari moja

11. Je nikiasi gani cha miti dawa unacho katika ghala yako?.....
12. Je baadhi ya miti dawa hii inauzwa nje ya nchi?.....
13. Kama ndio, unauza mwenyewe au watu wengine……..
14. Ni nchi zipi zinanunua mti dawa kutoka Tanzania?

    .....
15. Tafadhali nitajie miti dawa inayo safirishwa nje ya nchi

    ……
16. Ni mimea gani ni maarufu kwa wateja na inanunuliwa sana?

    …….....
17. Ni kitu gani kinafanya dawa hizo kua maarufu?

    ……
18. Unadhari umaarufu wa mimea dawa hiyo inaweza hatarisha upatikanaji wa mimeahiyo?

    ……kivipi(elezea)……
19. Je kunabaadhi ya miti dawa isiyo patikana tena katika sehemu za msingi huko inapo patikana?.....……..,kwa mda ganisasa

    ……...……
20. Nikitugani kimesababisha mimea hiyo kutoweka...…….……
21. Je ni kitu gani unafanya kuhakikisha uvunaji endelevu wa miti dawa?……
22. Je unakazi nyingine ukiacha ya kuuza dawa za asili? …….....
23. Je unafamilia (mke na watoto)……kama ndio,mkeo/motto anafanya kazi?.....
24. Kama ukipatakazi nyingine, ukiondoa ya kuuza dawa asilia; je utakubali…….
25. Tafadhali orodhesha miti dawa unayo uza na taarifa nyingine zitakazo hitajika katika jedwali hapa chini.

Mmea dawa	Uishipo C=umepandwa W=magugu Wi=porini	Sehemu ya mmea itumikayo *Fr= tunda* *Fl=ua* *L=majani* *SB=gamba* *SW=sehemu ngumu ya shina* *ST =shina lote* *RB=gamba la mzizi* *WR=sehemu ya ndani ya mzizi* *RT = mzizi wote* *WP=mmea wote* Nyingine	Mkoa	Njia ya uvunaji *U- kingoa* *BH-kuvuna gamba katika upana wake* *BV-kuvuna gamba katika urefu wake* *CW-kukata shina* *L- majani* *Fr- matunda* *Fl-maua*	Status *A- Inapatikana kiurahisi* *T-inapatikana japo kwa shida* *R-si rahisi kuipata* *E-haipatikani kabisa*	Disease treated

Shukrani kwa kushiriki

Appendix 5: List of the medicinal plants mostly exported from Tanzania.

Scientific names	Characteristic	Frequency	Percentage
Zanthoxylum chalybeum Engl.	Mjafari	19	15.57
Bauhinia thonningii Schum.	Kizabuni	11	9.02
Cassia abbreviata Oliv.	Mkundekunde	10	8.20
Zanha africana (Radlk.) Exell	Mdaula	6	4.92
Ximenia aegyptiaca L.	Mpingi	6	4.92
Bonamia mossambicensis (Klotzsch) Hallier f.	Mlipu	6	4.92
Rapanaea melanocephalus	Msaka uchawi,mpaja,msokonoi	5	4.10
	Kasela	5	4.1
Warburgia elongata Verdc.	Mwifu	4	3.28
Albizia anthelmintica Brongn.	Mfuleta,Mkutani	4	3.28
Ozoroa mucronata (Bernh.) R. Fern. and A. Fern.	Mhombe	3	2.46
Afzelia quanzensis Welw.	Mkongo	3	2.46
	Mkanda chuma	3	2.46
Sclerocarya birrea (A.Rich.) Hochst.	Mng'ong'o	2	1.64
Holarrhena febrifuga Klotzsch	Mmelemele	2	1.64
	Mgosihagona	2	1.64
	Nyota	2	1.64
Plectranthus amaniensis	Hoza	1	0.82
Eugenia caryophyllus (Spreng.) Bullock and S.G.Harrison	Karafuu	1	0.82
Tabernaemontana elegans Stapf	Umbombo	1	0.82
Spirostachys africana Sond.	Muharaka	1	0.82

*Senna didymobotrya (*Fresen.*)* H.S.Irwin and Barneby	Mwinu	1	0.82
Rapanaea sp.	Mlonda	1	0.82
ocimum americanus	Mwinula	1	0.82
Nigella sativa L.	Abat soda	1	0.82
Hymenaea verrucosa Gaertn.	Mkumbi	1	0.82
Hugonia arborescens Mildbr.	Nyambu	1	0.82
Ficus capensis Thunb.	Mkuyu	1	0.82
Curcuma longa L.	Manjano	1	0.82
Croton polytrichus Pax	Mlawa	1	0.82
Combretum fragrans F.Hoffm.	Mlama	1	0.82
Bombax rhodognaphalon K. Schum.	Mwale	1	0.82
Amaranthus spinosus L.	Kisakuanguku	1	0.82
Acacia xanthophloea Benth.	Mwita	1	0.82
	Kalialia	1	0.82
	Kalkari	1	0.82
	Kisokoza Wana	1	0.82
	Mbarikiwa	1	0.82
	Mdocteri	1	0.82
	Mkweati	1	0.82
	Mvule	1	0.82
	Mwengea	1	0.82
	Mzangazi	1	0.82
	Ngetwa	1	0.82
	Pekawake	1	0.82
	Ugufa	1	0.82
	Total	**122**	

Appendix 6: Medicinal plants identified through conventional method

Zanthoxylum chalybeum Engl.	Mjafari/ Msele/Mnungu/Rusuki/Mhunungu	56
Zanha africana (Radlk.) Exell	Mdaula/Mpanga	29
Cassia abbreviata Oliv.	Mkundekunde/Mkwizingwi/Ol Singwai/Mgama	29
Albizia anthelmintica Brongn.	Mfuleta/Ol Mukutani	28
Hymenaea verrucosa Gaertn.	Mkumbi/Ol Bukoi	24
Rapanaea melanocephalus	Mpaja/ Msaka Uchawi/ Sokonoi	21
Ximenia aegyptiaca L.	Mpingi/ Engamai	21
Bonamia mossambicensis (Klotzsch) Hallier f.	Mlipu/Mripo/Mrifu	20
Acacia xanthophloea Benth.	Ol Kiloriti/Mwita/Mkandekande	20
	Mkanda Chuma	16
Warburgia elongata Verdc.	Mwifu	15
*Sclerocarya birrea (*A.Rich.) Hochst.	Mng'ong'o	14
Combretum fragrans F.Hoffm.	Sandakusi/ Mlama/ Mkunguni	13
Suregada zanzibariensis Baill.	Mdimpori	12
Uvaria acrantha Miq.	Msofu	11
Holarrhena febrifuga Klotzsch	Mmelemele	11
Afzelia quanzensis Welw.	Mkongo	10
Acalypha ornata Hochst. ex A.Rich.	Mfulwe	10
Senna occidentalis (L.) Link	mwingajini	9
Piliostigma malabaricum (Roxb.) Benth.	ugue/ughue, mshegheshe	9
Artemisia afra Jacq. ex Willd.	pakanga,fivi	9
	kasela	9
Synaptolepis alternifolia Oliv.	msigi	8

Ozoroa mucronata (Bernh.) R. Fern. and A. Fern.	mhombe	8
Ficus sycomorus L.	mkunju	8
Tabernaemontana elegans Stapf	mbombo,neshekuye	8
Teclea nobilis Delile	ol rigilai	7
Senna didymobotrya (Fresen.) H.S.Irwin and Barneby	mwinu	7
Salvadora persica L.	msiganyika, ol remiti	7
ocimum americanus	mwinula	7
Ximenia americana L.	mtundwi	6
Ocimum basilicum L.	kivumbasi	6
Bauhinia thonningii Schum.	kizabuni	6
	mgosi hagona	6
Hugonia arborescens Mildbr.	nyambu	5
Ficus abelii Miq.	mvumo	5
Cissus integrifolia (Baker) Planch.	mtamba	5
Bombax rhodognaphalon K. Schum.	mwale	5
Abrus aureus R.Vig.	mfyambo	4
Chenopodium ambrosioides Loess.	muosha fedha	4
	mvuto	4
	mdindadinda	4
Strychnos spinosa Lam.	mtonga	3
Spirostachys africana Sond.	mwalaka	3
	londw/ilondwa	3
Pterocarpus angolensis DC.	mhagata	3
Ocotea usambarensis Engl.	kulo	3
Gardenia ternifolia Schumach. and Thonn.chumach. andThonn.	kilemela tembo	3

Fadogia elskensii De Wild.	kindukuli	3
*Dombeya rotundifolia (*Hochst.) Planch.	mtogo	3
Cissus acida L.	mwengele	3
Boscia salicifolia Oliv.	mguruka	3
Annona acuminata Saff.	mtomokwe/mtopetope	3
Amaranthus spinosus L.	kisakuakuku	3
Moringa oleifera Lam.	mlonge	3
Syzygium aromaticum (L.) Merr. and L.M.Perry	karafuu	3
Brackenridgea zanguebarica Oliv.	mkatakwa	3
	ol ndemwai	3
	mkongoraa	3
	mbarikiwa	3
Plectranthus amanuensis Gurke.	poza/hoza	3
	mkweyati/mkuyati	3
	mzangazi/mzanguzi	3
Ricinus communis L..	nyonyo	2
Pterocarpus mildbraedii Harms	mkula, kulo	2
*Pappea capensi*s Eckl. and Zeyh.	mhunguru	2
Nigella sativa L.	abatsoda	2
Foenugraecum officinale var.cultum Alef	uwatu	2
Ficus capensis Thunb.	mkuyu	2
Ehretia abyssinica R.Br. ex Fresen.	mjampofu/mjamofu	2
Dombeya acutangula Cav.	mjata, onjata	2
Cordia ovalis R. Br.	mkombati/mkombatele	2
Clausena anisata (Willd.) Hook.f. ex Benth.	mjavikali, zaituni	2
Clausena abyssinica Engl.	mkoma vikali	2
Chenopodium ambrosioides L.	ufunguo	2

Allophylus rubifolius (Hochst. ex A.Rich.) Engl.	msemelele	2
Alangium chinense (Lour.) Harms	mmavimavi	2
Adansonia digitata L	mramba	2
	magube	2
	pachori	2
	msio/mkete	2
Ocimum grantissimum L.	mlehani, mponda machili	2
Musa acuminata Colla	ndizi	2
	engiloito	2
	kasera	2
	dokiteri	2
Ehretia acuminata R.Br.	mkirika	2
	ugufa	2
Ziziphus jujuba Mill.	mkunazi	1
Zanthoxylum acanthopodium DC.	mnungunungu	1
Terminalia catappa	kungu	1
Telfairia pedata (Sm.) Hook.	kweme	1
Rhus natalensis Bernh. ex C.Krauss	Msambege	1
Pupalia lappacea (L.) Juss.	namata	1
Polysphaeria parvifolia Hiern	Mkame	1
Pericopsis angolensis (Baker) Meeuwen	uwanga	1
Osyris lanceolata Hochst. and Steud.	mdunula	1
Opilia amentacea Roxb.	lengurusai	1
Myrica adenophora Hance	kitalaswa	1
Loranthus acaciae Zucc.	mpingala	1
Lannea schweinfurthii Engl.	mumbu	1
Julbernardia globiflora (Benth.) Troupin	Mhangaa	1

Hoslundia opposia Vahl	mtulavua	1
Helianthus annuus L.	alizeti	1
Galiniera saxifraga (Hochst.) Bridson	mnengonengo	1
Elaeodendron afzelii Loes.	mcheka, mchekea	1
Diospyros fischeri Gürke	Mgoto	1
Dichrostachys cinerea (L.) Wight and Arn.	kikula gembe	1
Deinbollia kilimandscharica Taub.	Mtambakuzimu	1
Croton polytrichus Pax	mlawa	1
Croton macrostachyus Hochst. ex Delile	msinduzi	1
Combretum molle R.Br. ex G.Don	Chandama	1
Coffea arabica L.	kahawa	1
Carpolobia alba G.Don	mzukizuki	1
Carica papaya L.	papai	1
Bersama abyssinica Fresen.	Mbambaa	1
Balanites aegyptiaca (L.) Delile	mwamba ngoma	1
Asparagus acicularis F.T.Wang and S.C.Chen	msharifu	1
Albizia gummifera (J.F.Gmel.) C.A.Sm.	msama maji	1
Acacia sp.	usembe	1
Acacia nilotica (L.) Delile.	mkongowe	1
Hibiscus rosa-sinensis L.	rozela	1
	liwa	1
	manjano	1
	muegea	1
Ximenia caffra Sond.	muhingiro	1
	mkukusi	1
	mperuperu	1
	mtoe	1

	kalialia	1
Maesa lanceolata Forssk.	mgitahamu	1
	karoti pori	1
	kalibekantu, mtunguja	1
	kafadala,katila mwana	1
	lysugar	1
	mnyunyu	1
	songwi	1
	suza	1
	mpapatiko	1
	kausali	1
Ozoroa insignis	mwembe pori	1
	kiapo	1
	kitubai	1
	msausa	1
	mkaa pamoja	1
	magube	1
	abatimulki	1
	shubiri	1
Abrus precatorius L.	msipo	1
	kachili	1
	ebjani empukeli	1
	rekokola	1
	arapande	1
	oromatanga	1
	enduleley	1
	kisokozawana	1
	mwomboa	1

	alubatinuksi	1
	oromerorai	1
	onjasika	1
	onjorogera	1
	ondong'wai	1
	ndungulumi	1
	mkasio	1
	pekawake	1
Senna spp	salamaki	1
	mzalia nyuma	1
	mlosa	1
	aloevera	1
	Kipandipandi	1
	Mpondakaya	1
	Mderengwe	1
	Pilipili mwitu	1
	Mbashu	1
	Mbazo	1
	Mng'wanga	1
	Mwengakey	1
	Siu	1
Anisocycla blepharocephala Diels.	Ulenge	1
	Sagapa	1
	Friza	1
	Kibiriti upele	1
	Atang'oreng'ore	1
	Oliketaro	1
	zahatari(arab)	1

	kipa cha ibwe(pare)	1
	ola ng'orin'ori	1
	engitairo	1
	Fahari	1
	Kitar	1
	Girienyi	1
	Komesha	1
	ndimungoni	1
	Ulukukweyi	1
	gang’oro	1
	Mumelorayi	1
	Oalikoyi	1
	nkoponi	1
	Ngishekwi	1
	Singayi	1
	Tangawizi	1
	pilipili manga	1
	Janielibiliken,	1
	Muzizi	1
	Olikiu	1
Ocimum tenuiflorum	tulasi	1

Appendix 7: Medicinal plants that have other uses and transported for trade

Scientific name	local name	Mp value	other uses	Check point
Pterocarpus bussei Harms	Mninga	Yes	Timber	Vikindu
Afzelia quanzensis Welw.	Mkongo	Yes	Timber	Vikindu
Julbernardia globiflora (Benth.) Troupin	Mtondoro		Timber	Vikindu
Ochna densicoma Engl.	Msekeseke	Yes	Timber	Vikindu
Diospyros abyssinica (Hiern) F.White	Mkuruti	Yes	Timber	Vikindu
Milicia excelsa (Welw.) C.C.Berg	Mvule	Yes	Timber	Vikindu
Dalbergia melanoxylon Guill. and Perr.	Mpingo	Yes	Timber	Vikindu
	Pangapanga		Timber	Vikindu
Harungana madagascariensis Lam. ex Poir.	Mdamu Damu	Yes	Timber	Vikindu
Combretum schumannii Engl.	Mpera Mwitu			
Khaya nyasica Stapf ex Baker f.	Mkangazi(Very Rare Passes Here)		Timber	Vikindu
Polyscias fulva (Hiern) Harms	Mkongo	Yes	Timber	Vikindu
Suregada zanzibariensis Baill.	Mdimu Mpori	Yes	Building Poles	Vikindu
	Mlamba Mbulu			Vikindu
Ximenia americana L.	Mpingi	Yes	Charcoal	Vikindu
Senna siamea (Lam.) H.S.Irwin and Barneby	Mjohoro	Yes	Timber,Charcoal,	Vikindu
Prunus africana (Hook.f.) Kalkman	Mdundulungoma	Yes	Building Poles	Vikindu
Ocotea usambarensis Engl.	Mkulo	Yes		Vikindu
	Mtete		Building Poles,Charcoal,Poles	Vikindu
	Mburuzu		Building Poles,Charcoal	Vikindu
Amaranthus spinosus L.	Msakula Nguku/Msakula Mwale	Yes	Charcoal	Vikindu
	Minaki	Yes	For baskets Making	Kisarawe
Rauvolfia caffra Sond.	Mpugupugu	Yes		Kisarawe
Azanza garckeana (F.Hoffm.) Exell and Hillc.	Msembelezua	Yes	Indicator Species For Rain Season	Kisarawe
Hagenia abyssinica (Bruce ex Steud.) J.F.Gmel.	Mahanga	Yes	Indicator Species For Hunger.	Kisarawe
	Mtwini	Yes	Charcoal	Kisarawe

Appendix 8: Blast result of the samples sequenced

Collection no.venacular name		Scientific name	family name	Markers amplified	ITS1_17SE	ITS2_26SE
MP2	Salamaki	senna spp	Fabaceae	ITS	*Senna alexandrina*	
				ITS	*Senna alata*	
				ITS	*Senna italica*	
				ITS	*Senna auricilata*	
				ITS	*Senna hirsuta*	
MP3	Zalia nyuma	Phyllanthus amarus	Euphorbiacea	ITS	*Allophylus cobbe*	
				ITS	*Allophylus trichodesmus*	
				ITS	*Allophylus arboreus*	
				ITS	*Sapindus oligophyllus*	
				ITS	*Allophyllus longipes*	
MP23	Mkanda chuma			ITS	*Elaedondron Xylocarpum*	*Zanthoxylum schimfolium*
				ITS	*Brexiella cymosa*	*Zanthoxylum avicennae*
				ITS	*Brexiella spp*	*Toddalia asiatica*
				ITS	*Brexiella spp*	*Schisandrahenryi*
MP44	Mwinula	*Ocimum americanu*	Lamiaceae	ITS	*Ocimum basilicum*	*Ocimum basilicum*

		m				
				ITS	*Ocimum basilicum*	*Hyptis exserta*
				ITS	*Hyptis urticoides*	*Ocimum gratissimum*
				ITS	*Hyptis spp*	*Ocimum gratissimum*
				ITS	*Ocimum selloi*	*Occimum bascilicum*
				ITS	*Evonymopsis acutifolia*	*Platydesma spathulata*
MP52	Mkumbi	Hymenaea verrucosa	Fabaceae	ITS		*Toddalia asiatica*
				ITS		*Cleistanthus sumatranus*
				ITS		*Bridelia tomentosa*
				ITS		*Cleistanthus concinnus*
				ITS		*Bridelia affinis*
MP55	Mkongo	Afzelia quanzensis	Fabaceae	ITS	*Allophylus cobbe*	
		Landolphia parvifolia	Apocynaceae	ITS	*Allophylus arboreus*	
		Polyscias fulva	Araliaceae	ITS	*Allophylus trichodesmus*	
		Rauvolfia caffra	Apocynaceae	ITS	*Allophylus arboreus*	
		Balanites aegyptiaca	Balanitaceae	ITS	*Sapindus oligophyllus*	

		Commelina benghalensis	Commelinaceae	ITS	*Allophylus longipes*	
		Commelina africana	Commelinaceae	ITS	*Thouinia acuminata*	
MP59	Mudaura	Dialopsis africana	Sapindaceae	ITS		*Allophylus cobbe*
		Zanha africana	Sapindaceae	ITS		*Allophylus trichodes*
				ITS		*Allophylus arboreus*
				ITS		*Allophylus longipes*
				ITS		*Sapindus oligophylus*
MP65	Onjata			ITS		*Ximenia americana*
				ITS		*Symphytrichum laurentianum*
				ITS		*Domophyton anomalume*
				ITS		*Iphiona pinnatifida*
				ITS		*Symphytrichum undulatum*
MP77	Odongwai			ITS	*Prunus lusitanica*	*Knautia visiani*
				ITS	*Prunus azorica*	*Knautia velutina*
				ITS	*Crotalaria*	*Knautia*

					poisonii	*velebitica*
				ITS	*Crotalaria humbertiana*	*Knautia traunicensis*
				ITS	*Crotalaria axillaris*	*Knautia subscaposa*
MP83	Ugufa			ITS		*Abrus mollis*
				ITS		*Abrus precatorius*
				ITS		*Abrus mollis*
				ITS		*Abrus pulchellus*
				ITS		*Vatairea heteroptera*
MP87	Kisokoza wana			ITS		*Croton nobilis*
				ITS		*Croton manampetsae*
				ITS		*Croton goudotii*
				ITS		*Croton noronhae*
				ITS		*Croton klotzschianus*
MP89	Kisakua nguku	Amaranthus spinosus	Amaranthaceae	ITS	*Aristolochia sp*	*Croton argyratus*
				ITS	*Protea susannae*	*Croton chimboracensis*
				ITS	*Aglaia rufibarbis*	*Croton argyratus*
				ITS	*Trichosanthes*	*Croton*

					cucumerina	*purdiei*
				ITS	*Combretum padoides*	*Croton floccosus*
				ITS	*Combretum stylesii*	*Croton coriaceus*
MP111	Muharaka	Spirostachys africana	Euphorbiaceae	ITS	*Vouarana quianensis*	*Sedum surculosum*
				ITS	*Cupania diphylla*	*Ximenia americana*
				ITS	*Meineckia phyllanthoides*	*Symphyotrichum laurentianum*
				ITS	*Meineckia neogranatensis*	*Sedum jaccardianum*
				ITS	*Jacobaea carniolica*	*Kippistia suaedifolia*
				ITS	*Pilea carnulosa*	*Ilex ciliospinosa*
MP112	Mjampofu	Ehretia abyssinica	Boraginaceae	ITS	*Elaeodendron xylocarpum*	
				ITS	*Elaeodendron pininsulare*	
				ITS	*Elaeodendron cunninghanii*	
				ITS	*Celastraceae sp*	
				ITS	*Elaeodendron melanocarpum*	
MP121	Mponda machilI			ITS		*Holarrhena pubescens*
				ITS		*Funtumia african*

				ITS		*Callicarpa longissima*
				ITS		*Forsteronia leptocarpa*
				ITS		*Tabernamontana bufalina*
MP309	Hoza/poza	*Plectranthus amaniensis*	Lamiaceae	ITS	*Acacia alexandri*	
				ITS	*Acacia chartacea*	
				ITS	*Acacia ryaniana*	
				ITS	*Albizia julibrissin*	
				ITS	*Albizia kalkora*	
MP310	Msiganyika	Salvadora persica	Salvadoraceae	ITS	*Albizia kalkora*	*Cardiospermum schimperi*
				ITS	*Albizia julibrissin*	*Cardiospermum wightii*
				ITS	*Albizia julibrissin*	*Cardiospermum viminea*
				ITS	*Acacia synchronicia*	*Pistacia khinjuk*
				ITS	*Acacia ryaniana*	*Pistacia atlantica*
MP311	Kikulagembe	Dichrostachys cinerea	Fabaceae	ITS	*Allophylus cobbe*	
				ITS	*Allophylus trichodesmus*	
				ITS	*Allphylus arboreus*	

				ITS	*Sapindusoligophllus*	
				ITS	*Allophylus longipes*	
MP318	Mdocta			ITS	*Albizia julibrissin*	
				ITS	*Albizia kalkora*	
				ITS	*Acacia ryaniana*	
				ITS	*Acacia chartacea*	
				ITS	*Acacia synchronicia*	