APPLICATION OF TAXONOMIC AND DNA BARCODING TECHNIQUES IN IDENTIFYING COMMONLY TRADED HERBAL PLANT SPECIES IN SELECTED COUNTIES, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

To my children Hope Gathoni and Shawn Njomo and my nephew Nduba Kamiri who wishes to become a biologist.

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ACRONYMS AND ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Database
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
DNA	Deoxyribonucleic Acid
EDTA	Ethylene diamine tetra acetic Acid
GDP	Gross domestic product
GenBank	An online publicly available sequence database maintained by NCBI
GPS	Geographical positioning system
HPLC-MS	High Performance Liquid Chromatography_Mass Spectrometry
HTS	High-throughput sequencing
ISPs	Ion Sphere™ Particles
ITS	Internal transcribed spacer
IUCN	International Union for the Conservation of Nature
matK	Maturase K
MOTUs	Molecular Operational Taxonomic Units
NCB	National Center for Biotechnology
NGS	Next Generation Sequence
NCBI	National Center for Biotechnology Information
NMK	National Museums of Kenya
PCR	Polymerase Chain Reaction

PGM	Personal Genome Machine
rbcL	ribulose -1,5-bisphosphate carboxylase Large subunit
SRM	Standard Reference Material
TAE	Tris Acetic EDTA
TLC	Thin Layer Chromatography
trnL	Transfer RNA Leucine
WHO	World Health Organization
UI	Use Index
UV	Use Value

ABSTRACT

Over eighty percent of the world's population depends on herbal products for their basic health care needs. However, this widespread popularity is counterbalanced with the lack of relevant research to authenticate the source and purity of the traditional herbal products. There exists an extensive history on use of herbal products but lately there are concerns on authenticity and safety of these products. The objectives of the study were: determine plant species commonly traded as herbal products, assess the products using taxonomic and DNA barcoding techniques, application of DNA barcoding technology in reference barcodes generation, and determine if the plant herbal products are accurately labeled. The study was carried out in selected markets in three counties in Kenya (Kajiado, Narok and Nairobi). Data collection involved use of structured questionnaires, species observation, taxonomic and DNA barcoding techniques. Structured questionnaires were administered to individual herbalists and complementary interviews with key herbalists to enrich the data collected. The local and common names were recorded and later translated to scientific names using para-taxonomists and previously published data. Scientific names listed on labels of packaged plants products were as well recorded. The generated species list guided the collection of voucher specimens and creation of a reference library of DNA barcodes. DNA barcoding technology was used to authenticate the herbal product samples. The common single species samples were analyzed using Internal Transcribed Spacer *(ITS)* and ribulose -1,5-bisphosphate carboxylase Large subunit *(rbcL)* DNA barcoding markers while metabarcoding was applied in multi-species samples using nrITS2 marker. The study revealed that 86 plant species belonging to 43 families were traded as herbal products in the regions. Most of these plants were shrubs (66 %) traded as stem and bark. Majority of herbal plant species belong to Fabaceae, Apocynaceae and Rhamnaceae families. The DNA technology successfully generated barcode sequences that were used as reference for herbal products identification. Single species samples were found to be more authentic compared to the mixed species samples as most species were identified to species level as listed on the label. Adulteration in single species samples was mainly by substitution with closely related and/or looks alike species, which raises concern on value and quality of the herbal products sold in the studied counties. Kajiado County had more authentic samples in comparison to Narok and Nairobi. DNA metabarcoding technology was successful in identification of mixed species sample at 92 % to species level and 4 % each to genus and family level. This study has made the first attempt to identify herbal plant species traded in selected markets in Kenya using DNA barcoding technology in combination with morphological and literature methods. Authentication using DNAbarcoding provided a more reliable and accurate results compared to morphological identification. DNA barcoding should therefore be applied in identification and verification of herbal products. DNA barcodes successfully generated and deposited in National Centre for Biotechnology Information (NCBI) Genbank form crucial reference data for future studies and can be used as a baseline library for useful medicinal plants of Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Trade in herbal plant products is a flourishing commercial enterprise in global economy due to increasing demand in both developing and developed countries (Newmaster *et al.*, 2013). In the developing countries, the World Health Organization (WHO) documented that 65 % to 80 % of the population use herbal plant products as remedies to treat various ailments (WHO, 2011). The international trade on herbal products is on 15 % increase, with average annual revenue of at least US$60 billion (Srirama *et al.*, 2017). In many African countries trade in herbal plants form part of the informal economy contributing to the livelihood of the traders especially women (Jusu and Sanchez, 2014; Quiroz *et al.*, 2014). Herbal products demand has been increasing due to changes in cultural habits and beliefs that the products are natural and thus safe to use. Regulation of herbal products must be reinforced, particularly for the developing countries (Xin *et al*., 2015).

In Kenya, 80 % of the population is reported to use herbal remedies. The country is estimated to have forty thousand traditional healers including herbalists, bone setters, faith healers and birth attendants in 2011 (WHO, 2011). It is estimated that in Kenya alone, traditional medicine men utilize more than 260 plant species to treat numerous illnesses. For every three Kenyans, two of them depend on herbal products derived from plant parts or herbs as their primary source of health care (Bii Barnabas, 2018; Sifuna, 2022). Many patients use herbal products for pain relief and to treat long term illnesses (Peltzer and Pengpid, 2019).

Most people assume that herbal products are natural and safe to consume, thus non-toxic and with fewer adverse effects (Canter and Ernst, 2004b; Cohen and Ernst, 2010). Other reasons for their wide-spread use include ease of accessibility, affordability and personalized health care. Major uses of plant based remedies are health and wellness promotion and therapy for chronic diseases (Welz *et al*., 2018). However, the use of substandard quality, contaminated or counterfeit products poses health threat to the consumers. It is therefore vital to correctly identify the herbal plant species that are being used for production of traditional remedies for safe application.

The inability of users to visually confirm the identity of the herbal products additionally makes the scenario more problematic. Moreover, there lacks a structured approach to evaluate the safety and efficiency of these herbal remedies in most of the developing countries (Han *et al.*, 2016). The herbal products adulteration and substitution during supply chain coupled with unstandardized quality control during manufacturing can lead to substandard herbal products entering the marketplace and thus posing health risks to consumers (WHO, 2011).

Several challenges in identification of the plant species used in production of medicinal and herbal remedies have been reported (Ekor, 2014; Palhares *et al*., 2015; Yu *et al*., 2018; Ichim *et al*., 2020; Anthoons *et al*., 2021). Morphological characteristics and chemical analysis are inadequate in correctly distinguishing plant species used in herbal remedies. This is due to their possible absence of chemicals and flowers in and on the plant during most parts of the year (Da-cheng *et al*., 2010). Other methods like thin layer chromatography and high performance liquid chromatography-mass spectrometry have been used to authenticate the occurrence of target compounds but have limitations

in detecting substitutions and or additional plant species present but not listed on the label (Urumarudappa *et al*., 2016). This explains the need for other more powerful techniques such as the application of molecular techniques and DNA barcodes as a major step in screening the products used as herbal remedies.

DNA barcoding is the identification of species using standardized DNA fragments that are amplified and sequenced using universal primers following established protocols (Newmaster *et al.,* 2013). The technology has successfully been used previously in identification of undeclared species in crude drug trade of *Phyllanthus* (Euphorbiaceae), an important plant species vended as crude herbal drug for liver disorders treatment (Srirama *et al.,* 2010). DNA barcoding successfully differentiated *Phyllanthus* species and can therefore be utilized to analyze species mixtures in crude drug samples. Other studies on plant products in China such as Guo *et al*. (2011); Li *et al*. (2011); and Xue and Li (2011) also documented the scope and extent of species substitution in herbal products using DNA barcoding technology.

Molecular based techniques like Polymerase Chain Reaction (PCR), Polymerase Chain reaction restriction fragment length polymorphism (PCR–RFLP), DNA and oligonucleotide array analyses were previously used in phylogenetic and species identification (Tsumura *et al.,* 1995; Wei *et al.,* 2005). Despite their extensive use, the DNA barcoding methods have three intangible advantages over the mentioned methods: its universal since it uses one primer set across many species; its verifiability of identification by having voucher specimens; and the use of detailed nucleotide information that supports its identification (Jinbo *et al*., 2011).

DNA barcoding has been dubbed “a renaissance in herbal products identification” since it is an effective method for single species herbal medicine products identification, (Xin *et al*., 2015; Tnah *et al*., 2019; Newmaster *et al*., 2020). It is a reliable technique that does not require taxonomic expertise, is free from objective errors and gives substantial identification of obscure specimens (Abubakar *et al.*, 2017, 2018).

DNA barcoding methods are reliable techniques that will allow rapid identification of herbal plant products, their substitutes or derivatives and contaminants to their lowest taxonomic levels (genus and species) regardless of their age, part used, and/or plant habitat conditions (de Vere *et al*., 2012). The creation and the existence of DNA barcoding reference databases is a critical component for successful identification of mixed herbal products (Han *et al*., 2016; Tnah *et al*., 2019). Therefore, the application of DNA barcoding technique in quality evaluation of herbal products will bring into existence a cheaper, safer and reliable procedure, since the processors will promptly get rid of substitutions and contaminants without the need of undertaking expensive chemical analysis.

1.2 Statement of the problem

In the wake of increased population growth and increasing costs of living, majority of the people are seeking affordable medication and primary health care. This has resulted in an increased demand for herbal products and supplements that has led to over exploitation of natural resources. Newman and Cregg (2020) reckoned that more than 80 % of the world’s inhabitants depend on herbal products which is mainly plant based. It has been predicted that 10 to 80% of herbal products sold in developing countries are

adultered. In African countries, the adulteration of herbal drugs is estimated to be 80% due to lack of proper identification methods and techniques (Srirama *et al.*, 2017)

In Kenya, there are numerous bad effects on consumers after consumption of illegitimate plant based herbal remedies sold by medicine men (PSM, 2010). Counterfeit herbal contraceptives resulted to women giving birth to children who later displayed adolescence signs such as menstrual cycle at three years of age (PSM, 2010).

Most of the herbal products in the Kenyan market are packaged and labeled based on information from the herbalist and the sellers. Consequently herbal products available and being sold to customers in most of the markets are likely altered. They are replaced or altered with herbal products from look-alike or other plant species and fillers (Newmaster *et al.*, 2013). The herbalists do not disclose to the consumer what their products comprise besides the listed species. Studies by Newmaster (2013) showed mislabeling frequency of up to a third of the herbal products being sold in the markets that were adulterated or replaced with different or comparable plant species. The inability of consumers to visually confirm the identity of herbal products complicates quality checks.

Consumers of plant based herbal products should be guaranteed that the products they buy are safe for consumption and contain the listed species, pure or as a mixture of species. Consumers ought to be given recommended information on efficacy, dosage and possible negative impact (Ernst, 2005; Ribnicky *et al*., 2008). Provision of quality information is hindered by lack of a systematic approach to evaluate the quality, safety and efficacy of these remedies in most developing countries (Han *et al.*, 2016).

Herbal products may be contaminated and substituted due to misidentification (Shanmughanandhan *et al*., 2016). This is due to mix up with closely related plant species, accidental adulteration, and confusion resulting from local names use (Newmaster *et al*., 2013; Han *et al.*, 2016). Previous studies have shown discrepancies between species listed on products labels and those analyzed in the laboratory, thus raising concerns on herbal product quality and safety (Raclariu *et al*., 2017; Palhares *et al*., 2021; Anthoons *et al*., 2021). Therefore, there is the need for a dependable, robust, and cheap technique that will aid in accurate identification of plants constituting traded herbal products.

1.3 Justification of the study

Due to the growing consumer demand, potential toxicity and the market value of herbal products, there is need to give attention to production and marketing regulation of these products. Accurate identification of species in raw and processed herbal products and supplements is necessary to avoid health risks related to the presence of other poisonous plant species as well as ensure precise labelling and assessment of value (Han *et al*., 2016).

Therefore, it's essential to have better objective and scientific methods that can be used for individual species verification in herbal products. This way, the quality of herbal products can be evaluated and monitored. Molecular biology provides a collection of techniques that can be applied to confirm herbal plant products. DNA barcoding technology is one of the methods that has been used to verify plant species in herbal products (Li *et al*., 2011; Wallace *et al*., 2012)

There is therefore an urgent need for a robust, dependable and quality controlled method that will facilitate precise verification of herbal plant species in trade as listed on herbal product labels. In this study, the DNA barcoding technology was applied to authenticate herbal products traded in selected Kenyan markets for the benefit of protecting the indigenous ethnobotanical information and protection of humans from harmful herbal consumption.

1.4 Research questions

The following research questions were applied:

i. Which common plant species are traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties?
ii. Can taxonomic and DNA barcoding techniques identify the herbal plant species traded in selected markets in Kajiado, Nairobi and Narok counties?
iii. Is DNA barcoding technology able to generate reference barcode sequences to establish a library for common traded herbal plant species in selected markets in Kajiado, Nairobi and Narok counties?
iv. Are plant herbal products traded in the selected markets in Kajiado, Nairobi and Narok counties accurately labeled?

1.5 Hypotheses

i. There are common plant species traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties.

ii. Common plant species traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties can be identified using taxonomic and DNA barcoding techniques.

iii. DNA barcoding technology can be used to generate reference barcodes for library creation for common traded herbal plant species in selected markets in Kajiado, Nairobi and Narok counties.

iv. Plants herbal products traded in selected markets in Kajiado, Narok and Nairobi counties are accurately labeled.

1.6 Objectives

1.6.1 General objective

To map out common plant species traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties and generate reference DNA barcodes library

1.6.2 Specific objectives

i. To determine common plant species traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties.

ii. To assess common plant species traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties, using taxonomic and DNA barcoding techniques.

iii. Assess application of DNA barcoding technology in reference barcodes generation of traded herbal plants common in selected markets in Kajiado, Nairobi and Narok counties.

iv. To determine if plant herbal products traded in selected markets in Kajiado, Nairobi and Narok counties are accurately labeled using DNA and metabarcoding technology.

1.7 Significance of the study

This study has successfully identified useful herbal plant species utilized as herbal products. The information can be applied in domestication and commercialization of herbal products with the aim of increased utilization. The study has also proved the usefulness of DNA barcoding in authentication of herbal products traded. The results of this study can be applied in development of herbal products regulation. In addition, consumers are advised to purchase herbal products from credible herbalists.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter reviews literature on; utilization of plants in health care, global trends in herbal products and practice, safety regulation in herbal products, conventional identification of herbal plant products and the utilization of DNA barcoding in confirmation of herbal products.

2.2 Utilization of plants in health care

Since early days of humanity, plants have been used for ailments treatment and promotion of good health all over the world (Pan *et al.*, 2014). Plants and other natural sources contribute majorly to the commercial medicine preparations manufactured today. At least 25 % of the currently prescribed drugs worldwide are obtained from plants (Wachtel-Galor and Benzie, 2011). Herbs are preferred more than synthetic drugs and are frequently used in health care systems. Some people prefer herbal products as their main method of cure while others use them as supplementary therapy to convectional prescriptions.

In the past self-health care has been on increase as most consumers have chosen to take charge of their individual wellbeing. Majority of the consumers prefer traditional herbal products and practices, with the assumption that, “natural” means “safe” which is not certainly true (Ekor, 2014). The potential of cost savings is a significant motive for persons to opt for herbal products. For example, a study in the recent past recorded that patients who received health advice from general physician who had alternative

medicine complimentary training have lower mortality rate and low health upkeep costs compared to those who have none (Howland, 2020). Low prices of herbal products compared to conventional medicine makes people to prefer herbal products.

2.3 Global use of herbal products

Herbal products consumption is one of the ancient methods of treatment recognized and used by all races. Globally, herbal products use is preferred by many and it is considered to offer an alternative treatment for numerous ailments, predominantly lifestyle diseases that require lifetime pharmaceutical medication. In the past, herbal products have been considered save for disease prevention and alternative to conventional medicine (Newman and Cragg, 2016). It is practiced in developed as well as developing countries by both patients and medical practitioners. In Europe it is estimated that over 70 % of medical doctors from France and Germany regularly prescribe herbal products to their patients, in China herbal products forms 30 % to 50 % of their total drug uptake and in United States, about 158 million people use herbal products (Khan and Ahmad, 2019).

Globally, the market value for herbal products currently stands at over US$ 60 billion per annum with growth rate of 7 % (Gunjan *et al*., 2015). Herbal products are traded raw or processed (Lange, 2006).

2.4 Herbal products use in Kenya

In Kenya, over 70 % of the population rely on herbal medication as their primary healthcare requirements (Kisangau and Herrmann, 2007). The traditional herbal specialists ratio to the overall population is greater than that of doctors of allopathic or conventional medicine in an identical population (Howland, 2020). However, effective application and utilization of herbal remedy is hampered by the fact that there lacks an implementation strategy or regulatory framework upon which medicine men and herbalists operate. The demographic categorization of herbal practitioners in Kenya has recognized that more women than men are engaged in administering herbal products (Kipkore *et al*., 2014, Okumu *et al*., 2017). Various cadres of specialization in herbal products that are recognized in Kenya are described in Table 2.1.

Table 2.1: Specialization groups within the herbal medicine practice in Kenya

Specialization	Skill set
Herbalist	Concoct medicines from plant parts as well as other materials. Treat respiratory, digestive and urinary conditions as well as intestinal parasites
Generalist	Use plant products but are not considered specialists for any specific diseases
Dentists	Equipped with knowledge of particular plant products with tooth relieving properties. They also extract decayed/ broken teeth
Traditional birth attendants	Middle aged/older women with vast experience and knowledge in all herbs related to reproduction
Bone setters	Manage bone ailments, malformations using herbal medicine
Spiritual/faith healers	Relieve stress, depression, mental disturbances by use of herbal remedies

Adopted from World Health Organization (2005) and Okumu et al. (2017).

In the last fifteen years, there has been an increase in research and publications on herbal plant-use in Kenya. For example Jeruto *et al*. (2008) documented the herbal plants of the Nandi forest, Okello *et al*. (2010) documented the indigenous information on herbal plants of Mt. Elgon forest, Ndegwa (2008) documented herbal plants utilized by the Ogiek community, and Ochwang'i *et al.* (2014) investigated herbal plants used to treat cancer in Kakamega forest. Recently, herbal plants among the Marakwet have been investigated by Kipkore *et al*. (2014) and the herbal plants used by Loita Maasai have been reported by Nankaya *et al.* (2020).

However, one of the major gaps identified in these studies was validation and identification of plant species used by local herbalists and their formulations. Majority of the herbal products being sold in markets at present are lacking any scientific proof of identification, efficiency and safety (Bent, 2008). Furthermore, several non-governmental organizations have ventured into the herbal products trade, either to extract raw materials for conventional medicine (Njoroge, 2002) or to protect local indigenous knowledge (Swiderska, 2006). As a result, the knowledge generated is pushing for the establishment and enforcement of a legal framework for formulation and use of herbal plants (Okumu *et al*., 2017).

There seems to be inadequacies in tackling the challenges of herbal medicine practice in the country (Okumu *et al*., 2017). For instance, misidentification of plants and herbs used in the formulation of medicine can only be overcomed if the legal and regulatory framework of herbal products identification and validation is put in place. The consequences of non-regulation of the herbal products industry have resulted in dwindling of botanical resources as a result of over exploitation, urban expansion and

over population (Chen *et al.,* 2016). The harvesting of herbal plants has resulted to destruction of forests and plants, some of which are of critical importance.

The herbal products industry further suffers from substitutions and contaminations of herbal herbs with other closely associated plant species (Techen *et al*., 2014; Ichim *et al*., 2020) because there is no systematic identification and documentation of herbal plant product. As a consequence, the usefulness of these drugs decreases if it is contaminated and in some cases can be poisonous if it is replaced with lethal contaminants. Therefore, for the herbal products to be effective, their accurate formulation is key.

2.5 Regulation of herbal products practice in Kenya

The regulation of herbal medicine practice in Kenya is still a major challenge (Okumu *et al*., 2017; Ichim *et al*., 2020) and its implementation is done by different institutions. The Ministry of Social Services is responsible for registration of herbalists in the country. The commercially formulated and packaged herbal products are registered by the Kenya Pharmacy and Poisons Board (PPB). However, the indigenous knowledge on herbal products is protected by 1994 convention on biodiversity (Kimball, 1995). Although there are institutions that govern use of herbal products, there are no policies that guide in the production and conservation of these herbal plant species.

2.6 Safety of herbal productss

Herbal products have previously been used to prevent and cure diseases as well as to boost life quality. Many people consider herbal products to be safe since they are

extracted from natural plants (Ekor, 2014). The products are frequently sold either as single species or as mixtures. The herbal plant species are sourced from different geographical regions. These herbal plants contain various biological active ingredients (Canter and Ernst, 2004a; Qato *et al*., 2008; Loya *et al*., 2009; Cohen and Ernst, 2010).

There is limited data on the constitution and quality of herbal products in the market since there is lack of well-established government guidelines. In addition, there is no acceptable research methods for assessing herbal products (WHO, 2000; Kantor, 2009). Further, comprehensive list of species for most of the herbal products is not reported on the label; neither do the herbalists share this information with the buyers. Consequently, it becomes problematic to know the species used in production of these herbal products and their safety. Thus, the consumers raise health concerns as a result of lack of herbal products use safety assurance.

The high demand of herbal products use in diseases management has resulted to increasing concern in regards to their safety and quality. The suspected toxicity and unwanted side effects may be due to adulteration, overdoses and contamination that may arise during processing (Mensah *et al.*, 2019; Hasen and Hashim, 2021). Some of the actions that can be taken to ensure herbal products quality standards are maintained include: reliable botanical identification, quality packaging and storage.

2.7 Conventional identification of herbal plants products

The methods used in the past for herbal plant identification include organoleptic approaches (identification through the senses: taste, smell, touch, sight), microscopic and macroscopic approaches (identification by color, texture, shape,) and chemical profiling (such as HPC-MS, HPLC-UV and TLC) (Lindegårdh *et al*., 2007; Han *et al*.,

2016). Nevertheless, all these methods are not able to differentiate closely related species in processed products. Verification at DNA level offers more dependable results because DNA is a stable macromolecule present in all tissue and not affected by environmental factors (Yu *et al*., 2016).

2.8 Application of DNA barcoding in identification of plants

A DNA barcode is a portion of the genome (marker) found in a broad range of species (Kress, 2017). Barcoding requires small tissue sample from a plant or animal for identification. It can also be used in cases where specimen is processed or degraded.

The standardized barcode for plants is a fragment of the plastid gene ribulose 1, 5-biphosphate carboxylase gene (*rbcL*) combined with a fragment of the maturase K (*matK*) gene of chloroplast which is highly conserved in plant systematics. The choice for the *rbcL* is grounded on easy amplification recovery while that of *matK* is based on its discriminatory power. One of the most fast evolving coding segment of the plastid genome is *matK* although it can be problematic to amplify for some groups within angiosperms (Hollingsworth *et al*., 2011). The gene contains high substitution rates within the species and is emerging as potential candidate to study plant systematics and evolution. The *rbcL* barcode region is easy to amplify in most of the plant and its sequencing success rate is also high. It provides valuable barcode dataset besides having lower discriminatory power compared to *matK* (Hollingsworth *et al*., 2011).

Translation of sequences generated using *matK* and *rbcL* markers to amino acid is done to check for pseudogenes, editing and assembly errors as well as to confirm correct sequence orientation as both markers are coding regions Hollingsworth *et al*., 2011.

The data generated has a coding and alignable nature that allows direct character based and diversity analysis among different taxonomic groups and also comparison of samples from different geographical regions (Flynn *et al*., 2015; Okoth *et al*., 2018).

Internal Transcribed Spacer (*ITS2*) is a potential nuclear DNA barcode that is easy to amplify and sequence (Zhao *et al.*, 2018). It has shown high discrimination ability among herbal plant species and their close relatives (Chen *et al.*, 2010; Yao *et al.*, 2010). It has been proposed as a standard DNA barcode marker for herbal plants authentication by researchers due to its high variability and power to determine species (Zhang *et al.*, 2018).

The nuclear ribosomal internal transcribed spacer 2 (nrITS2) marker has been recorded to have shorter read length of previous high-throughput sequencing platforms, but the marker is more variable and has a greater potential for identification at higher taxonomic levels than the shorter and less variable Transfer RNA Leucine (trnL) P6 loop (Veldman *et al.*, 2017). The higher level of sequence variation is important for identification of closely related herbal plant species. Several metabarcoding studies using nrITS1 or nrITS2 for species identification of herbal plants have been reported (Cheng et al., 2014; Coghlan et al., 2012; De Boer et al., n.d.; Raclariu et al., 2017).

DNA barcoding sequencing involves Sanger sequencing or next generation sequencing (metabarcoding). Sanger sequencing is usually applied for identification of single species since it processes only a single DNA fragment at a time. The assumption is that if a product contains a single species, the fragment generated from Sanger sequencing will efficiently identify that species. Moreover, Next generation sequencing (metabarcoding) processes millions of DNA fragments simultaneously at a time

allowing detection of variations within related species (Mohrbeck *et al.*, 2015; Crossley *et al.*, 2020). Metabarcoding is able to deduce all species in a mixed species sample.

The Ion Torrent system is unique among Next Generation sequencing (NGS) technologies because the detection of sequencing is not based upon fluorescent dyes, but measures pH changes as the result of the release of a H^+ ion (Egan *et al.*, 2012). There are two systems that use NGS technology, the Ion PGM and Ion Proton. The PGM sequencer can produce over 1000 Mb of sequence with 11.1 million sensors and the system has clearly made its way into studies pursuing plant-based identification (Egan *et al.*, 2012).

The Ion Torrent PGM sequencing has two amplification steps, one for library preparation and the other emulsion PCR (emPCR) for template amplification. The library preparation step involves fragmenting genomic DNA and attaching specific adapter sequences (Egan *et al*., 2012; Quail *et al*., 2012). The unique -MIDx tags are attached to the forward while uniform -trP1 tags are attached to reverse Ion Torren PGM primers respectively. DNA fragments are linked with specific adapter sequences and amplified by emulsion PCR on the surface of 3μm diameter beads. Sequencing is primed from a specific location in the adapter sequence. Each of the four bases is introduced sequentially, protons are released and a signal is detected proportional to the number of bases incorporated (Quail *et al*., 2012).

The sanger sequences generated can be assembled using Geneious software version 11 (Kearse *et al.*, 2012). The primer sequences are trimmed from both the forward and complementary strands in order to retain a high-quality target DNA sequence region (Crossley *et al*., 2020).The forward and the reverse sequences are then aligned to

produce a contig. The contig sequence is blasted against reference sequences in NCBI for species identification. Further confirmation can be done by a neighbor joining tree generation using the same software.

Barcoding techniques have previously been used in: testing herbal products (Stoeckle *et al*., 2011; Sui *et al*., 2011; Wallace *et al*., 2012), verification of plant derived products such as teas, spices, olive oil, and characterization of plant origins of honey (Asahina *et al*., 2010; Heubl, 2013; Pang and Chen, 2019). Other studies using barcoding techniques by Palhares *et al.* (2015), Srirama *et al.* (2010) Techen *et al.* (2012)and Wallace *et al.* (2012) reported high frequency (71 %) of plant species adulteration, and 14 % to 33 % substitution rate.

The technique can further be used for detection and identification of CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) (https://cites.org) listed herbal plants to help in the control of illegal trade (Dalton and Kotze, 2011; Xue and Li, 2011; Gathier *et al*., 2013). Barcoding involves DNA based identification and is changing from single species to multiple species metabarcoding from a complex sample through high-throughput DNA sequencing (Cristescu, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter highlights the research methods used in the study. It presents the research design, sampling procedures, data collection methods (both qualitative and quantitative) and data analyses.

3.2 Description of the study area

The study was carried out in selected markets in three counties i.e. Kajiado, Narok and Nairobi in Kenya where herbal products consumption and trade is of significant proportions (Bussmann *et al*., 2006; Mwangi and Gitonga, 2014). Kajiado and Narok counties were selected for this study because they are inhabited by Maa community who rely on herbal products as their primary source of heath care (Mwangi and Gitonga, 2014). Nairobi County was identified because it neighbors Kajiado and offers market for herbal products from the other two counties. The study site and markets where data was collected are as shown in Figure 3.1 while Table 3.1 summarizes selected market centers.

Kajiado County lies in the Southern end of the Kenyan Rift Valley and borders Tanzania in the Southwest. The County lies at the rain shadow of Mount Kilimanjaro and has a semi-arid climate (Campbell *et al.*, 2000; MoALF, 2018). The vegetation is dominated by bushland, grassland and open woodlands along the seasonal river valleys. The total population in Kajiado is 1,117,840 inhabitants (KNBS, 2019), who mainly belong to the Maa community. The local inhabitants are believed to have a strong

cultural and traditional orientation and rely on herbal products from plants collected from both Kenya and Tanzania.

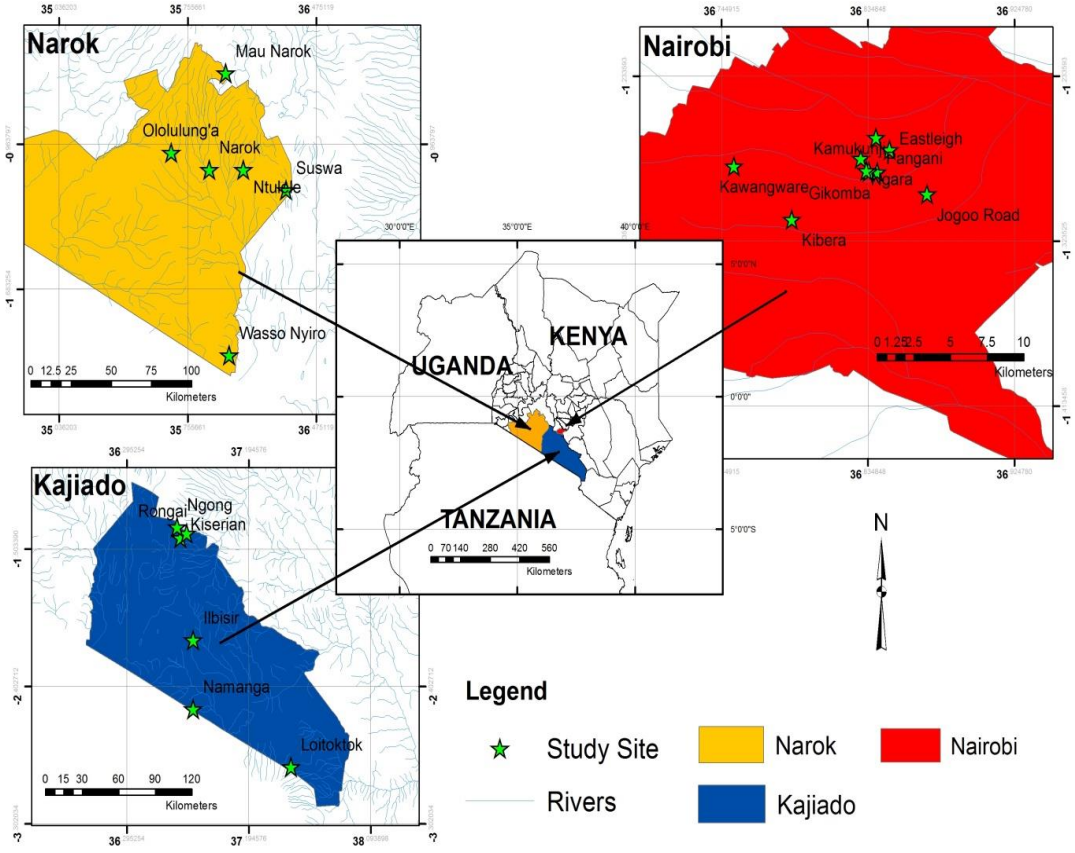

Figure 3.1: Main map showing Kenya and the locations of the counties - Narok, Nairobi and Kajiado. Insert maps outlines the counties and markets selected for data collection. Source: Mwaura *et al.* (2020).

Narok County is located along the Great Rift Valley and borders Tanzania country to the South. The County has a population of 901,777 (KNBS, 2019) and hosts the Mau forest complex and Loita forest. The largest closed-canopy forest ecosystem in Kenya is formed by the Mau Forest Complex. It is the most significant watershed in the Rift Valley and Western regions of Kenya. Loita forest is predominantly semiarid with dry upland forest and extensive grasslands of approximately 330 km^2 which lies between

the Nguruman - Magadi escarpment and the Maasai Mara National Game Reserve. The forest is rich in plant biodiversity and has great cultural and religious worth to the indigenous communities. The forest has been reasonably well maintained but due to increasing demand for forest products, land for cultivation and grazing biodiversity has been on the decline (Kariuki *et al*., 2016). The two forests harbor high biodiversity of herbal plant species believed to be a source of primary health care for the Maa and other neighboring communities.

Nairobi is a metropolitan County that hosts Nairobi town, the capital city of Kenya with a population of 4,397,073 (KNBS, 2019), and contributes 60 % of the country's GDP. It thus hosts a big clientele for herbal products (Bussmann *et al*., 2006; National Coordinating Agency for Population and Development, 2008; Mwangi and Gitonga, 2014).

3.3 Sampling procedures and data collection

3.3.1 Selection of sampling sites and identification of informants

The study areas were purposively selected to include diverse locations and markets where plants of herbal value are sourced or traded (Table 3.1). Market centers were randomly selected from each study County within 25 Km from main urban centre as people within that area can easily access the markets. In each County, a representative sample of 30 respondents who were practicing herbalists ware selected. The target population of respondents consisted of herbalists domiciled in Kenya and trading herbal products. There is a research gap in registration of herbalists in Kenya and therefore the actual population of herbalist's is not known (Okumu *et al*., 2017).

Therefore, a total sample size of 90 herbalists was arrived at as a conservative figure to represent the three counties.

Table 3.1: Selected counties, market centers and number of herbalists sampled

County	Market centre	No of herbalist sampled
Narok	Wasso Nyiro	6
	Suswa	6
	Narok Town	5
	Ntulele	5
	Mau Narok	5
	Ololulunga	3
Nairobi	Eastleigh	2
	Gikomba	2
	Jogoo road	5
	Machakos Country Bus	3
	Kibera	5
	Ngara	4
	Kamukunji	3
	Kawangware	3
	Pangani	3
Kajiado	Kiserian	4
	Rongai	1
	Ngong	4
	Namanga	7
	Ilbisir	9
	Loitoktok	5
	Total	**90**

3.3.2 Data collection methods

Data collection involved application of both qualitative and quantitative techniques. Structured questionnaires were administered to individual herbalists in order to maintain data independence (Appendix I) while complementary ethnobotanical methods including species observation and interviews with key herbalists were used to

enrich the data collected The markets sampled were based on popularity of herbalists and only willing herbalists were interviewed. Mixed-species samples contained more than one plant species.

3.3.3 Ethno-botanical survey

To document the plant species traded as herbal products, ethno botanical field surveys and laboratory analysis were carried out between 2017 and 2021. Semi-structured interviews, group discussions and direct observations (Appendix II) were used to gather ethno pharmacological information from herbalists trading at selected open air markets after obtaining prior informed consent. The respondent's bio-data was recorded and maintained to ensure no re-sampling from same herbalists as most of them operate in more than one market. Other meta data recorded include: herbal plants used (common name and/or scientific names), purity of the products (single or mixture of species), uses and plant part used for formulation (leaves, bark, roots, fruits, flowers, and tubers), registration status of herbalists (either registered or has permits) and clinical conditions treated were recorded.

3.3.4 Taxonomic analysis of historical data

Secondary data sources included analysis of taxonomic information to examine plant uses and verification of scientific names which was used to enrich the primary data. The local or common names were recorded and later translated to scientific names by para-taxonomist and taxonomists at the East African Herbarium - National Museums of Kenya and in reference to published data (Maundu *et al.,* 2001; Kokwaro, 2009).

3.4 Sampling of herbal products

Herbal products including raw and processed materials available or sold in selected markets were sampled from interviewed herbalists and additional samples were obtained from supermarkets within study site and used for molecular identification and authentication. The samples were packaged in self-sealing bags, labeled and transported to the National Museums of Kenya Molecular laboratory.

3.4.1 Field sampling for reference plant samples

Species were selected based on a generated list from the field market survey and identified in the field by plant taxonomists. Herbarium voucher specimens were collected (as much as possible collected with either flowers or fruits), using standard botanical procedures, labeled and pressed in the field (Appendix III). They were later quarantined, accessioned and stored at East African herbarium (EA) at the National Museums of Kenya (NMK). Further identification and validation were carried out using the applicable taxonomic keys at the East African herbarium at the National Museums of Kenya (Beentje *et al*., 1994; Agnew, 2013).

Five specimens of fresh leaf samples were individually collected from identified species in the wild, labeled and stored in paper envelopes containing silica gel for preservation and transported to the laboratory for further DNA analysis and archival. Taxonomists from the East African Herbarium were engaged to identify the listed reference species in the field. These samples were later used to generate barcodes to create the reference library together with other available barcodes. For any species that had look - a - like

species, collection of the look - a - like was also done. Species from the same genus that were referred using the same local names were also collected where possible.

Both the voucher specimen for herbarium and leaves for DNA analysis were collected and images with taxonomic characteristics taken. Other metadata recorded included geographical locations, GPS points, species identification confidence, identifier and date of collection. In the laboratory, the leaf tissues were subsampled to barcoded tubes and assigned tissue barcodes for archival while a second subsample was used for analysis. The archival tissues are currently stored at NMK, Molecular Genetics Laboratory for future reference. All the field data and generated tissue barcodes were recorded and managed using Field Information Management System (FIMS) for ease of use and flexibility of operation, (Deck *et al*., 2012)

3.5 DNA barcoding

3.5.1 DNA extraction and gel electrophoresis

The total genomic DNA was extracted from 0.1g of the dry leaf material using the DNeasy® plant mini kit (Qiagen®) according to the manufacturers' instructions with some modifications for market samples. In brief, the dry material was ground using a Genogrinder (Model 2010) to obtain a fine powder, and then 400 µl of Buffer AP1 which is a lysis buffer and 4 µl RNase A was added. The mixture was vortexed for one minute and incubated for 120 minutes instead of the recommended manufacturer's 60 minutes at 65 °C in a shaking water bath. After incubation, 130 µl of buffer P3 which is a neutralizing buffer was added, mixed and incubated for 5 minutes on ice. The lysate was centrifuged through the kit column and precipitated DNA cleaned. The elution of

recovered DNA was carried out in 100 µl of elution buffer. The DNA eluted in buffer was stored in -20 ° C during analysis. For reference collected samples, DNA was extracted as per manufacturers' protocol. Mixture samples analyzed using metabarcoding were extracted using silica-based extraction (Rohland and Hofreiter, 2007).

Agarose gel electrophoresis procedure was carried out to confirm presence and quality of extracted DNA. One percent agarose was prepared by dissolving 1 g of agarose powder in 100 ml of 1X TAE (Tris Acetic EDTA) buffer and cast on a mound with combs for forty five minutes. Five microliters of extracted DNA sample was mixed with 2 µL of 6X orange loading dye and loaded in each well. A 100 bp DNA ladder (Magbio) was included as standard for the electrophoresis process. The gel was run at 100 volts for 30 minutes and the recovered purified DNA products were visualized in the UV trans-illuminator and photographed in Gel Doc system (EnduroTM GDS Gel Documentation System). The quality and concentration of the genomic DNA recovered was determined using spectro-biophotometer (Model X200) at 260/280 nm.

3.5.2 DNA amplification using Polymerase Chain Reaction (PCR)

The amplification of the positively extracted DNA for reference and market single species samples was carried out using a Bioneer lyophilized PCR ready mix in a 20 µL reaction mixture that contained 2.0 µL of 10 ng genomic DNA, 1 µL each of forward and reverse primers (10 pM); and 16 µL deionized nuclease free PCR water.

PCR reaction was performed using Nexus Eppendorf master cycler. The *matK* and *rbcL* barcode loci for coding genes as well as *ITS* were amplified. The primer sequence and

amplification conditions are as stipulated in Table 3.2. Success of amplification was confirmed by loading 3 µL of amplified product in 1.5 % agarose gel and run for 30 minutes at 80 volts against a 100 bp DNA ladder. Samples with multiple bands were diluted and re-amplified to increase and improve specificity while the faint samples, DNA template were increased during amplification.

3.5.3 PCR product purification

The positive amplified fragments that had only single bands were purified using ZYMO DNA clean and concentrator kit (Catalogue no. ZR D4034), as per manufacturers' protocol. In brief, 1:7 volume of binding buffer was mixed with the amplified product. The resultant mixture was passed through the kit column and centrifuged for the amplicon to bind to the filter. Two washes with the kit provided ethanol wash buffer were carried out and then eluted in a 100 µl of elution buffer. The success of purification was confirmed in 1.5 % agarose gel electrophoresis. The concentration of purified product was measured using a spectro-biophotometer was measured at 260 / 280 nm.

Table 3.2: Primer sequences and cycling conditions used in the study

	Forward Primer: Sequence 5' and 3'	**Reverse Primer: Sequence 5' and 3'**
Primer name	*rbcL*a_F - ATGTCACCACAAACAGAGACTAAAGC (Kress and Erickson, 2007)	*RbcL*R590 – AGTCCACCGCGTAGACATTCAT (de Vere *et al.*, 2012)
Cycling conditions	Initial denaturation step- 5 min at 94°C,35 cycles of denaturation 30 sec at 94 °C, annealing at 56 °C for 30 sec, extension for 40 sec at 72 °C. Final extension at 72 °C for 10 min.	
Primer name	*matK*_1R_kim: ACCCAGTCCATCTGGAAATCTTGGTTC (K. J. Kim, unpublished)	*MatK*_3F_Kimr: CGTACAGTACTTTTGTGTTTACGAG (K.J. Kim, unpublished)
Cycling conditions	Initial denaturation step -2 min at 95 °C, 39 cycles of denaturation 30 sec at 95 °C, annealing at 58 °C for 1 min 30 sec, extension for 45 sec at 72 °C. Final extension at 72 °C for 5 min	
Primer name	ITS 2F - ATGCGATACTTGGTGTGAAT (Chen *et al.*, 2010)	ITS3 R – GACGCTTCTCCAGACTACAAT (Chen *et al.*, 2010)
Cycling conditions	Initial denaturation step- 5 min at 94 °C, 35 cycles of denaturation 30 sec at 94 °C, annealing at 55 °C for 30 sec, extension for 35 sec at 72 °C. Final extension at 72 °C for 10 min.	

3.5.4 DNA sequencing

Purified PCR products from reference voucher samples and the single species market samples were subjected to Sanger sequencing. All Sanger sequencing was done at Macrogen Inc., Netherlands. All positive purified products of collected voucher specimen with a concentration of above 20 ng / µl and those of single species samples from the market were sequenced. The sequences for voucher specimens were submitted as reference library. The amplification primers were used as the sequencing primers. For mixed-species samples, the amplicons underwent next generation sequencing using Ion-Torrent PGM (De Boer et al., n.d.; Veldman et al., 2017).

3.6 Barcode reference library creation

A barcode reference library was prepared for frequently traded herbal plants and their close relatives where possible. The library was assembled from plant species of known origin that were identified by taxonomic experts and their respective herbarium voucher specimens archived in the East African Herbarium - National Museums of Kenya. This reference library was used to identify the species of herbal products from the sampled markets.

The Field Information Management System (FIMS) was used to manage field meta-data and linked to laboratory data using Laboratory Information Management Systems (LIMS) provided by NMK. For most species, at least five DNA barcodes from different geographic regions were generated. The *matK, rbcL* and *ITS* sequences were edited and aligned using Geneious software Version R11 to produce barcode contigs. To assemble the forward and reverse sequences to produce a contig, De novo assembly was selected, then selected merge 1st part of name, separated by underscore. The Highest

Sensitivity/Slow option was selected since the sequences were below 1000bp. The save assembly report, save list of unused reads, save in sub-folder and save contigs were selected. The translation of consensus sequence was done in highest quality threshold, correct open reading frame (1-3) was selected and genetic code for plants translation (trans_table_11) to ensure no stop codons were present in contig and the sequence was aligned in forward orientation. The final consensus sequences were deposited in the Barcode of Life Database (BOLD) and NCBI GenBank.

3.7 Single species herbal product barcode samples identification and analysis

Herbal plant products of single species were analyzed in triplicates per marker using *rbcL*, *ITS* and *matK* plant barcoding markers (de Vere *et al*., 2012) with an aim of using at least 2 markers of identification. Multiple DNA extracts of these samples were barcoded to make sure all species' DNA present was detected. This allowed identification of samples that possibly consisted of a mixture of species.

Identification of unknown barcode sequences from sampled herbal products sold in the markets was carried out by comparing Basic Local Alignment Search Tool (BLAST) data against the barcodes from the generated barcode reference library with a minimum BLAST cut off of 97 % identity for a top match (Altschul *et al*., 1990). The results were verified by analyzing neighbor-joining tree branches of query specimens to those of reference species sequences.

3.8 Mixed species herbal products metabarcoding identification

A significant amount of herbal products in the sampled markets are sold as mixtures, thus metabarcoding was used to differentiate and identify the species present in such samples.

The study analyzed seventy two mixed species samples out of which only 62 samples had positive quantifiable genomic DNA. Extracted DNA was amplified using the nuclear ribosomal internal transcribed spacers nrITS2 using primers 5.8I2 and 26SE, respectively (Sun *et al*., 1994). To increase identification success of all species present in the mixture sample, all the samples were amplified in triplicates. The adapters MIDx tags (unique) and trP1 uniform tags were used to label the Ion Torrent Personal Genome Machine (PGM) forward and reverse primers respectively.

The PCR was done in 25 µl total reaction volumes, that contained: 5 µl Phire 5X PCR buffer, 5 µl 5x TBT-PAR (Samarakoon *et al*., 2013) 1 µl of 0.25 µM of each primer, 1 µl of 10 mM dNTP, 0.5 µl of 4 U/ µl Phire hot start Taq polymerase, 8.5 µl of milliQ (Ultrapore) pure water and 3 µl of template DNA.

The following PCR protocol as described by Veldman *et al*., (2017) was used: Firstly, 40s of initial denaturation at 98 °C, followed by 35 cycles (denature at 98 °C for 5 s, annealing at 50 °C for 20 s and elongation at 72 °C for 15 s) and a final elongation step of 60 s at 72 °C. After the amplification, amplicons were purified and selected with 0.99 Agencourt AMpure beads as per laid down standard protocol. An equimolar pool concentration of the eluted amplicons was done through measurement on a Bioanalyser using a DNA 1000 chip (Agilent). Equimolar pools prepared were then measured on the Bioanalyser with a high sensitivity chip and diluted according to the calculated

template dilution factor to target 10 to 30 % of all positive Ion Sphere™ Particles (ISPs).

The template preparation of this pool was carried out on an Ion One Touch instrument with the Ion PGM Template OT2 400 kit (Life Technologies) according to Manual 7218 v3.0. The ion sphere quality control kit was used to check the quality of the Ion One Touch2 400 ion sphere particles on a Life Qubit 2.0. The ion spheres were loaded on a 314 chip v2 and next generation sequenced with the ion PGM sequencing 400 kit (Life Technologies) on an Ion-Torrent personal genome machine (Life Technologies, ThermoFischer Scientific, USA). The output was analyzed using the High-throughput sequencing (HTS) barcode checker (Lammers *et al*., 2014) to infer the different species present in each mixture.

3.9 Data analysis

3.9.1 Quantitative analysis

The ethnobotanical data generated from interviewed herbalists was analyzed using diverse quantitative indices including Use Value (*UV*) and percentage Use Index (% UI). The data was entered into Excel spreadsheet, cleaned using Google Refine and summarized using descriptive statistics (Hoft *et al*., 1999). The data was then filtered to determine frequencies of citations and responses in order to identify the most common families and species in the study areas, popularly used as herbal plants, their uses and plant parts used and traded.

3.9.2 Use value (UV)

Use value (*UV*) determines the relative importance on uses of plant species (Phillips and Gentry, 1993; Krupa *et al*., 2019). In order to assess the respondent's valuation of the herbal plant species, use-value indices were calculated as follows:

$$UV_i = \frac{\sum Ui}{N} \quad \text{.....1}$$

Where *UV* indicates use value of individual species, "*U"* is the total number of uses recorded for that plant species and *"N"* is the total number of herbalists interviewed in each County. A species that records high *UV* score indicates there are many recorded uses in ailments treatment as cited by interviewed herbalists (Faruque *et al*., 2018). The herbal plant use combinations mentioned by the interviewees were counted. The informants were interviewed only once therefore each plant-disease-use combination mentioned by each informant was interpreted as an event. Theoretically, *UV* varies from *0* to *1* where *0* denotes that none of the informants mentioned any use of the plant while *1* implies that the plant is most often cited as useful in treatment of the highest number of ailments.

3.9.3 Percentage Use Index (%UI)

For every herbal species recorded, a Use Index (*UI %*) was calculated to give the importance of use and trade of the species as reported by respondents across the three counties under study.

The percentage Use Index *(% UI*) is derived from the formula:

$$\% \text{ UI} = \frac{na}{NA} * 100 \quad \text{.....2}$$

Where: *na* is the number of herbalist who were selling that particular species at the time of sampling; *NA* is the total number of herbalists that were interviewed.

3.9.4 Sequence data analysis

The *matK*, *rbcL* and *ITS* were sequenced using Sanger sequencing method. Sequence data generated was assembled and analyzed using Geneious software R11 (Kearse *et al*., 2012) to generate barcodes. TranslatorX software was used for alignments of the protein coding regions for *rbcL* and *matK* sequences. BLAST against reference sequences in NCBI as well as generating phylogenetic trees was done using consensus sequence using Geneious software. The partial *ITS*, *rbcL* and *matK* reference nucleotide data were deposited in the GenBank with a 'barcode' tag.

For metabarcoding, FASTQ read files were processed using HTS-barcode-checker pipeline (Lammers *et al*., 2014) following Galaxy settings under Naturalis Biodiversity Centre work flows (Https://galaxy.naturalis.nl). The nrITS2 primer sequences were used to de-multiplex the sequencing reads per sample and to filter out any reads that did not match any of the primers using the HTS pipeline. PRINSEQ (Schmieder and Edwards, 2011) was used to define filtering and trimming values based on read lengths and Phred read quality (Ewing *et al*., 1998). All reads with a mean Phred quality score of below 26 were filtered out, as well as those reads with a length of below 200 bp. The remaining reads were trimmed to a maximum length of 450 bp.

The CD-HIT-EST (Li and Godzik, 2006) was used to cluster reads into Molecular Operational Taxonomic Units (MOTUs) that was defined to consist of a minimum number of 2 reads and a sequence similarity of at least 99 % (Edgar, 2013, 2016;

Ryberg, 2015; Rognes *et al*., 2016). The resultant consensus sequences of non-singleton MOTUs were queried using BLAST (Altschul *et al*., 1990) against a reference nucleotide sequence database, with a maximum e-value of 0.05, a minimum hit length of 100 bp and sequence identity of at least 97 %. The reference sequence database consisted of a local copy of the NCBI/GenBank nucleotide database generated from *nrITS* sequences in NCBI GenBank at the time of analysis. The number of reads per MOTU, as well as the BLAST results per MOTU, were compiled using custom scripts from the HTS Barcode Checker pipeline (Lammers *et al*., 2014; Veldman *et al*., 2017).

3.9.5 Authentication classification

The terms and definition that were used when assessing identity of the herbal products sampled from the market were adopted from Newmaster *et al.*, (2013) and are outlined below:

i. Authentic: A product was authentic if it contained DNA barcode sequences for species that were its main species on the label of the tested product.

ii. Contamination: A product was contaminated if a DNA barcode sequence was found for a species other than what was labeled on the tested product in addition to the authentic barcode. This included known product filler species.

iii. Substitution: Product substitution was realized when a DNA barcode sequence was found for a species other than what was labeled on the tested product, and there was NO barcode sequence recovered for the main species listed. This strict definition did not consider whether it was an accidental mis-identification of a bulk product or a fraudulent market substitution for a cheaper product.

iv. Filler: A product contained fillers if a DNA barcode was found for known herbal product filler species such as rice (*Oryza sativa*), soybean (*Glycine max*) and wheat (*Triticum spp*). Fillers may be found in place of (substitution) or in addition to the barcode of the main product species (contamination).

For single plant species product, a sample was considered authentic if the listed or given name matched scientific name as identified by blast hits produced by *ITS* and *rbcL* contigs whereas *MatK* was used to resolve any discrepancies. A minimum identification identity of 97 % and an e-value of 0.0001 were considered for a top match. As long as the species was identified by either of the markers it was recorded as present

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents an analysis of research findings per specific study objectives. The chapter first addresses the social landscape of the respondents and a synthesis of plant species commonly traded as herbal remedies in selected markets in Kenya. The chapter further presents the finding of the specific objectives which are to (a) determine common plant species traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties, (b) assess common plant species traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties, using taxonomic and DNA barcoding techniques, (c) assess application of DNA barcoding technology in reference barcodes generation of traded herbal plants common in selected markets in Kajiado, Nairobi and Narok counties and (d) determine if plant herbal products traded in selected markets in Kajiado, Nairobi and Narok counties are accurately labeled using DNA and metabarcoding technology.

4.2 Herbal plant species traded in selected Kenyan markets

A total of 86 plant species were traded in the study area as single species. Most of the recorded species as reported by herbalists belong to the family Fabaceae (17 species) followed by Apocynaceae, Rhamnaceae and Rutaceae with four species each (Figure 4.1). Sixteen out of 43 families recorded had at least two species being traded in the three counties and they were considered as the dominant families in this study.

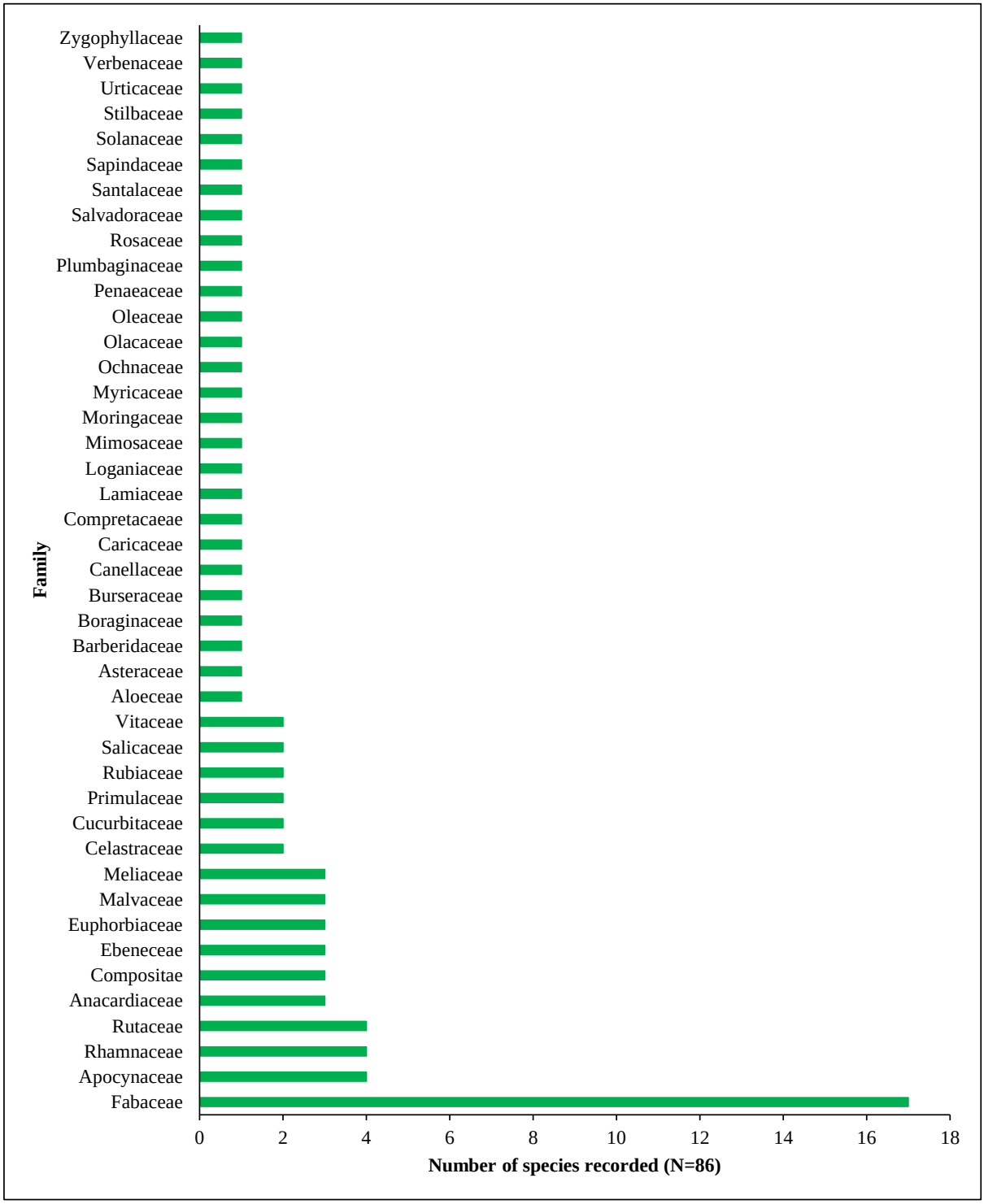

Figure 4.1: Families of herbal plants traded in the three study counties (Narok, Kajiado and Nairobi) and respective species count based on herbalists' reports.

4.3.1 Taxonomic classification of herbal plant species in the study counties

The study revealed that a total of 86 herbal plant species belonging to 43 floristic families from the 940 single product entries (N = 940) recorded by herbalists were traded in the study counties based on parataxonomy and published literature (Table 4.1). The study revealed that Kajiado had the highest number of plant species (71) belonging to 43 families that were traded and used as herbal remedies. There were 63 species belonging to 41 families in Narok County and 55 species belonging to 37 families in Nairobi County, which were traded as herbal plants respectively. Out of the 86 species recorded, 44 species were common across the three selected counties and belong to 31 families based on taxonomic identification.

Table 4.1: List of ethnomedicinal plant species traded in selected markets in Nairobi, Kajiado and Narok counties (pages 42 to 48)

S.No	Family	Species and Voucher ID	Plant part used	County where the herbal plant was recorded
1.	Aloeceae	*Aloe spp*	Leaves	Narok, Nairobi, Kajiado
2.	Anacardiaceae	*Ozoroa insignis,* Delile (NMK: 10894)	Stem	Narok
3.	Anacardiaceae	*Schinus molle,* L. *(NMK:133980)*	Stem, roots	Nairobi, Kajiado
4.	Anacardiaceae	*Searsia natalensis, (*Bernh. ex C. Krauss) F.A.Barkley (NMK:10747)	Stem, seeds	Narok, Nairobi, Kajiado
5.	Apocynaceae	*Acokanthera schimperi, (*A. DC.) Benth. & Hook. f. ex Schweinf. (NMK:10779)	Stem	Narok, Nairobi, Kajiado
6.	Apocynaceae	*Carissa edulis,* Forssk. (NMK:10794)	Stem, bark, leaves, roots, seeds	Narok, Nairobi, Kajiado
7.	Apocynaceae	*Mondia whytei,* (Hook. f.) Skeels (NMK:10330)	Stem, roots	Nairobi
8.	Apocynaceae	*Periploca linearifolia,* Quart.- Dill. and A.Rich. (NMK:10900)	Stem	Narok, Nairobi, Kajiado
9.	Asteraceae	*Psiadia punctulata,* Vatke (NMK:10728)	Stem, bark, roots	Narok, Nairobi, Kajiado
10	Asteraceae	*Gutenbergia cordifolia Benth. ex Oliv. (NMK:13575)*	Leaves	Kajiado
11.	Barberidaceae	*Berberis holstii, Engl.* (NMK:10789)	Stem, roots, leaves	Narok, Kajiado
12.	Boraginaceae	*Commiphora africana, (Rich.)*	Bark	Kajiado

S.No	Family	Species and Voucher ID	Plant part used	County where the herbal plant was recorded
		Engl (NMK: 13576).		
13.	Burseraceae	*Commiphora swynnertonii, Burtt (NMK: 13577)*	Leaves	Nairobi
14.	Canellaceae	*Warburgia ugandensis, Engl.* (NMK:13005)	Stem, bark, leaves	Narok, Nairobi, Kajiado
15.	Caricaceae	*Carica papaya, L. (NMK:13392)*	Stem, bark	Narok, Nairobi
16.	Celastraceae	*Maytenus senegalensis, (Lam.) Exell (NMK:13367)*	Stem, bark, leaves, roots, seeds	Narok, Nairobi, Kajiado
17.	Celastraceae	*Mystroxylon aethiopicum, (Thunb.) Loes (NMK: 13410)*	Bark, leaves, roots, seeds	Narok, Nairobi, Kajiado
18.	Compositae	*Solanecio angulatus, (Vahl) C. Jeffrey (NMK:13578)*	Bark	Narok, Kajiado
19.	Compositae	*Tarchonanthus camphoratus, L. (NMK:13579)*	Stem,bark	Narok, Kajiado
20.	Compositae	*Vernonia brachycalyx, O. Hoffm.* (NMK:13008)	Stem, bark, leaves, roots	Narok, Nairobi, Kajiado
21.	Compretacaeae	*Combretum molle, R. Br. ex G. Don* (NMK:10802)	Stem, roots	Narok, Nairobi, Kajiado
22.	Cucurbitaceae	*Momordica friesiorum, (Harms) C.Jeffrey (NMK:13580)*	Stem, bark, leaves, roots, seeds	Narok
23.	Cucurbitaceae	*Zehneria scabra, (L. f.) Sond.(NMK:13581)*	Stem	Kajiado
24.	Ebeneceae	*Euclea divinorum, Hiern* (NMK:10809)	Stem, bark, leaves, roots, seeds	Nairobi, Narok, Kajiado
25.	Ebeneceae	*Euphorbia candelabrum, Trémaux ex Kotschy*	Stem	Nairobi

S.No	Family	Species and Voucher ID	Plant part used	County where the herbal plant was recorded
26.	Ebeneceae	*Euphorbia sp.*	Stem	Nairobi
27.	Euphorbiaceae	*Croton dichogamus, Pax (NMK:13341)*	Stem, bark, leaves, roots, seeds	Narok, Nairobi, Kajiado
28.	Euphorbiaceae	*Croton megalocarpus, Hutch. (NMK:13382)*	Stem, leaves	Kajiado
29.	Euphorbiaceae	*Croton somalensis, Vatke and Pax ex Pax (NMK:13558)*	Stem, bark, leaves	Narok, Nairobi, Kajiado
30.	Fabaceae	*Acacia abyssinica, Hochst. ex Benth. (NMK:13335)*	Stem, bark	Kajiado
31.	Fabaceae	*Acacia drepanolobium, Harms ex Y.Sjöstedt* (NMK:10971)	Stem, roots	Narok, Kajiado
32.	Fabaceae	*Acacia gerrardii, Benth. (*NMK:10774)	Stem, bark, roots, seeds	Nairobi, Kajiado
33.	Fabaceae	*Acacia mellifera, Vahl. (NMK: 13514)*	Stem, bark	Narok, Nairobi, Kajiado
34.	Fabaceae	*Acacia nilotica, Schumach. and Thonn* (NMK:10775)	Stem, bark, roots, leaves, seeds	Narok, Nairobi, Kajiado
35.	Fabaceae	*Acacia nubica, Benth. (NMK: 13390)*	Stem, bark, roots, leaves, seeds	Narok, Nairobi, Kajiado
36.	Fabaceae	*Acacia Senegal, (L.) Willd. (NMK:13377)*	Stem, bark	Nairobi, Kajiado
37.	Fabaceae	*Acacia thomasii, Harms (NMK:1313583)*	Stem, bark	Kajiado
37.	Fabaceae	*Acacia tortilis, Forssk. (NMK:13364)*	Stem, bark	Nairobi
39.	Fabaceae	*Acacia xanthophloea, Benth.* (NMK:10776)	Stem	Kajiado

S.No	Family	Species and Voucher ID	Plant part used	County where the herbal plant was recorded
40.	Fabaceae	*Albizia amara, Boiv.* (NMK:13010)	Stem, bark	Narok, Nairobi, Kajiado
41.	Fabaceae	*Albizia anthelmintica, Brongn. (NMK:13394)*	Stem, bark	Narok, Nairobi, Kajiado
42.	Fabaceae	*Albizia schimperiana, Oliv.(NMK:13584)*	Stem	Kajiado
43.	Fabaceae	*Caesalpinia volkensii, Harms. (NMK:13585)*	Bark	Kajiado
44.	Fabaceae	*Ormocarpum trachycarpum, (Taub.) Harms (NMK:13586)*	Stem	Kajiado
45.	Fabaceae	*Piliostigma thonningii, (Schum.) Milne-Redh. (NMK:13554)*	Stem, bark	Narok, Nairobi, Kajiado
46.	Fabaceae	*Pterolobium stellatum, (Forssk.) Brenan (NMK:13587)*	Leaves	Narok, Kajiado
47.	Lamiaceae	*Ajuga remota, Benth. (*NMK:10770)	Bark	Nairobi, Narok, Kajiado
48.	Loganiaceae	*Strychnos henningsii, Gilg (NMK:10849)*	Stem, bark,leaves	Narok, Nairobi, Kajiado
49.	Malvaceae	*Grewia bicolor, Juss. (*NMK:10741)	Stem, bark, leaves, roots, seeds	Narok, Nairobi, Kajiado
50.	Malvaceae	*Grewia similis, K.Schum. (NMK:10985)*	Stem, bark	Narok
51.	Malvaceae	*Hibiscus flaviflorus, (F. Müll.) Kuntze (NMK:13345)*	Stem, bark	Narok
52.	Meliaceae	*Azadirachta indica, A.Juss. (NMK:13418)*	Stem, bark, leaves	Narok, Nairobi, Kajiado
53.	Meliaceae	*Turraea abyssinica, Hochst.* (NMK:10867)	Stem, bark leaves	Narok, Nairobi, Kajiado

S.No	Family	Species and Voucher ID	Plant part used	County where the herbal plant was recorded
54.	Meliaceae	*Turraea mombassana, C. DC.* (NMK:10871)	Stem, bark	Narok, Nairobi, Kajiado
55.	Mimosaceae	*Rapanea melanophloeos, (L.) Mez (NMK:13548)*	Seeds,	Narok, Nairobi, Kajiado
56.	Moringaceae	*Moringa oleifera, Lam. (NMK:13437)*	Stem, leaves	Nairobi
57.	Myricaceae	*Myrica salicifolia, Hochst. Ex A.Rich (NMK: 13560).*	Stem, bark	Narok, Nairobi, Kajiado
58.	Ochnaceae	*Ochna ovata, F.Hoffm. (NMK:13588)*	Stem, bark, leaves, roots, seeds	Narok
59.	Olacaceae	*Ximenia Americana, L.* (NMK:10865)	Stem, bark, leaves, roots	Narok, Nairobi, Kajiado
60.	Oleaceae	*Olea europaea, L. (NMK:*10984)	Stem, Bark	Narok, Nairobi, Kajiado
61.	Penaeaceae	*Olinia rochetiana, A.Juss.* (NMK:10872)	Stem	Narok, Kajiado
62.	Plumbaginaceae	*Plumbago zeylanica, L. (NMK:13564)*	Stem, bark, leaves, roots	Narok, Nairobi, Kajiado
63.	Primulaceae	*Embelia schimperi, Vatke* (NMK:10805)	Stem, bark, leaves, roots, seeds	Narok, Nairobi, Kajiado
64.	Primulaceae	*Myrsine Africana, L.* (NMK:10980)	Stem, bark	Narok, Kajiado
65.	Rhamnaceae	*Rhamnus prinoides, L'Hér* (NMK:13019)	Stem, bark	Narok, Nairobi, Kajiado
66.	Rhamnaceae	*Rhamnus staddo, A.Rich* (NMK:13015)	Stem, bark, leaves, roots	Narok, Nairobi, Kajiado
67.	Rhamnaceae	*Scutia myrtina, (Burm.f.)* Kurz (NMK:1010831)	Stem, roots	Narok, Kajiado

S.No	Family	Species and Voucher ID	Plant part used	County where the herbal plant was recorded
68.	Rhamnaceae	*Ziziphus mucronata, Willd. (NMK:12385)*	Bark, root	Nairobi
69.	Rosaceae	*Prunus africana, (Hook.fil.) Kalkm. (*NMK:10329*)*	Stem, bark	Narok, Nairobi, Kajiado
70.	Rubiaceae	*Tarenna graveolens, (S.Moore) Bremek. (NMK:13589)*	Stem,bark	Narok
71.	Rutaceae	*Toddalia asiatica, (L.) Lam.* (NMK:13014)	Stem, bark, leaves, roots	Narok, Nairobi, Kajiado
72.	Rutaceae	*Vepris nobilis, (Delile) Mziray* (NMK:10852)	Stem,	Kajiado
73.	Rutaceae	*Vepris simplicifolia, (Engl.) Mziray* (NMK:10860)	Stem, bark, leaves, roots	Narok, Kajiado
74.	Rutaceae	*Zanthoxylum usambarense, (Engl.) Kokwaro* (NMK:10968)	Stem, Bark	Narok, Nairobi, Kajiado
75.	Salicaceae	*Dovyalis abyssinica, Warb.* (NMK:11019)	Stem	Narok, Kajiado
76.	Salicaceae	*Trimeria grandifolia, (Hochst.) Warb.* (NMK:10851)	Stem, bark, roots	Narok, Nairobi, Kajiado
77.	Salvadoraceae	*Salvadora persica, L (NMK:13590)*	Stem, bark, roots	Narok, Nairobi, Kajiado
78.	Santalaceae	*Osyris lanceolata, Hochst. and Steud.* (NMK:13001)	Stem, bark, leaves, roots, seeds	Narok, Nairobi, Kajiado
79.	Sapindaceae	*Pappea capensis, Eckl. and Zeyh*. (NMK:10872)	Stem, bark, leaves, roots, seeds	Narok, Nairobi, Kajiado
80.	Solanaceae	*Withania somnifera, (L.) Dunal (NMK:13591)*	Leaves, roots	Narok, Kajiado

S.No	Family	Species and Voucher ID	Plant part used	County where the herbal plant was recorded
81.	Stilbaceae	*Nuxia congesta, R. Br. (NMK:13592)*	Stem, bark	Narok
82.	Urticaceae	*Urtica massaica, Mildbr. (NMK:13593)*	Leaves, roots, stem	Narok, Nairobi, Kajiado
83.	Verbenaceae	*Clerodendrum myricoides, (Hochst.) R. Br. ex Vatke (*NMK:10801)	Stem, bark, leaves, roots, seeds	Narok, Nairobi,Kajiado
84.	Vitaceae	*Cissus quadrangularis, L. (NMK:13594)*	Stem, bark,	Kajiado
85.	Vitaceae	*Rhoicissus tridentate, (L .f.) Wild and R. B. Drumm.* (NMK:10913)	Stem, bark	Narok, Nairobi, Kajiado
86.	Zygophyllaceae	*Balanites aegyptiaca, Delile (*NMK:10743)	Bark, roots	Nairobi

4.3.2 Plant growth form of traded herbal species in the study area based on sampled products

Majority of the herbal plant life form identified in this study were shrubs (66 %) followed by trees (16 %), herbs (12 %) and climbers (6 %) (Figure 4.2).

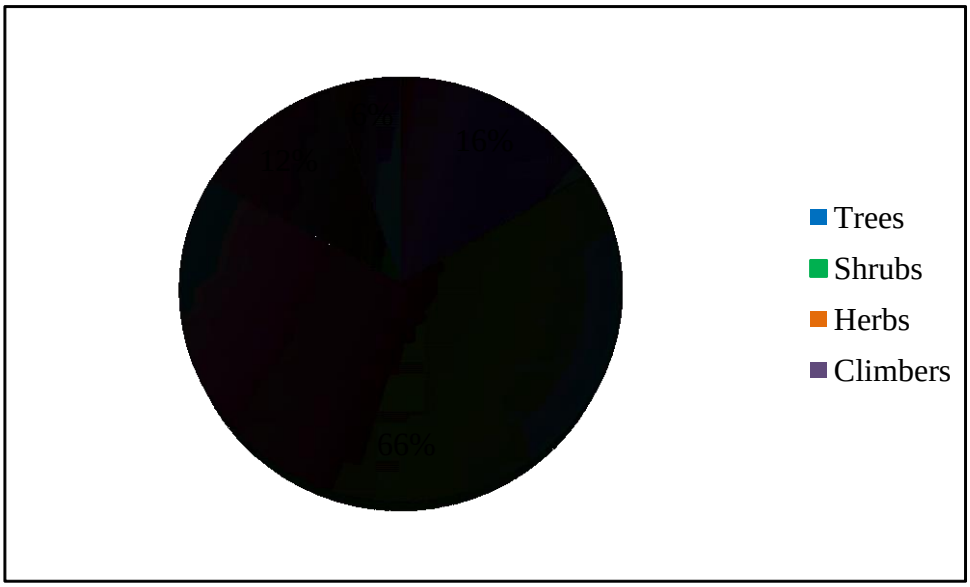

Figure 4.2: Different growth forms of herbal plants identified in the study area.

4.3.3 Herbal plant parts commonly traded by herbalists in the study area

Even though all plant parts are used in preparation of herbal remedies, the present study revealed that stem, bark, leaves, roots, seeds and tubers were used as herbal remedies either alone or in combinations. Stems were the most commonly utilized and traded plant part with 34 % followed by barks (33 %) and roots (13 %). The stems were the most commonly traded plant parts in Kajiado and Narok counties at 34 %, while in Nairobi, barks were the most traded plant parts (35 %) (Figure 4.3).

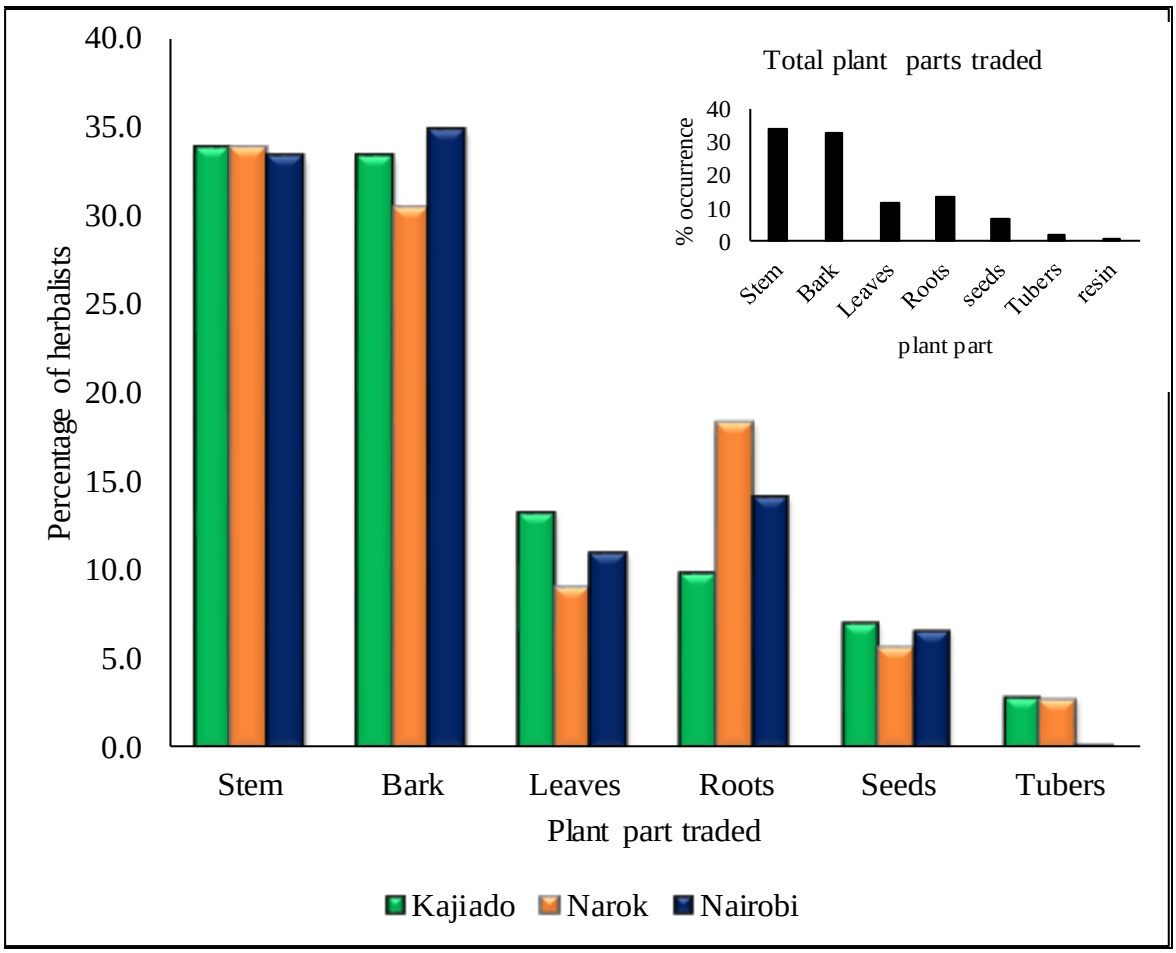

Figure 4.3: Percentage of plant parts citations used by herbalists. Inset: Total of the plant parts traded for herbal use in Narok, Kajiado and Nairobi counties.

While most herbal plant species traded were common in all the three study regions, a few plant species were specific to the sites as shown in Table 4.2. Such plant species were only traded in the specific counties. Kajiado had the highest number of unique species (12) followed by Nairobi (8) and Narok (7).

Table 4.2: Plant species traded as herbal remedies which were specific to the studied counties

Kajiado	Nairobi	Narok
Acacia thomasii	*Carica papaya*	*Grewia similis*
Cissus quadrangularis	*Euphorbia candelabrum*	*Ozoroa insignis*
Zehneria scabra	*Balanites aegyptiaca*	*Momordica friesiorum*
Acacia abyssinica	*Commiphora swynnertonii*	*Tarenna graveolens*
Acacia xanthophloea	*Ziziphus mucronata*	*Hibiscus flaviflorus*
Albizia schimperiana	*Moringa oleifera*	*Nuxia congesta*
Caesalpinia volkensii	*Acacia tortilis*	*Ochna ovata*
Commiphora africana	*Mondia whitei*	
Croton megalocarpus		
Guttenbergia carviflorus		
Ormocarpum trachycarpum		
Vepris nobilis		

4.3.4 Herbal plant species and treatment associations

The study revealed that plant species were associated with ailments that could be treated or managed by the plant product. The reported disease categories or ailments were classified into 14 categories based on use reports (Table 4.3). The three prevalent ailments in the study regions were abdominal, respiratory and malaria and were treated by the highest recorded species at 18.8 %, 14.3 % and 13.5 % use reports respectively. Urinary disorders had the least use reports in this study. The study revealed that the most common herbal plants being traded in the markets are for treating health conditions including respiratory and abdominal pains.

Table 4.3: Summary of common disease ailments treated by herbal products in Nairobi, Kajiado and Narok counties as reported by herbalists

Treatment category	Ailment	Number of plant species (use reports) (frequency)	Percentage of use reports (%)
1.	Abdominal	25	18.8
2.	Respiratory diseases	19	14.3
3.	Malaria	18	13.5
4.	Sexual diseases	15	11.3
5.	Nutrition	15	11.3
6.	Body aches	14	10.5
7.	Body energizer	6	4.5
8.	Fertility	6	4.5
9.	Snake bites	4	3.0
10.	Skin disorders	3	2.3
11.	Eye infections	3	2.3
12.	Nervous disorders	2	1.5
13.	Dental	2	1.5
14.	Urinary disorders	1	0.7
	Total	**133**	**100**

4.3.5 Use index and use value of herbal plants in the study area

Results of use index and use value of the herbal plants in study area showed that *Osyris lanceolata* had the highest percentage use index (68.9 %), followed by Rhamnus *staddo* (54.4 %), *Rapanea melanophloes* (53.3 %) and *Acacia nilotica* (52.2 %). *Clerodendrum myricoides and Combretum molle* had the highest use value of 0.12 followed by *Euclea divinorum* (0.11) and *Warburgia ugandensis, Searsia natalensis*, and *Maytenus senegalensis* at 0.10 respectively. *Mystroxylon aethiopicum, Aloe sp, Acokanthera schimperi, Vernonia Brachycalyx, Periploca linearifolia, Turraea abyssinica* had the lowest use value at 0.01 (Table 4.4). .

Table 4.4: Common herbal single species traded in the three counties, their percentage Use Index (UI), Use Value (UV) and their treatment associations (pages 53 to 57)

S. No.	Scientific Name	Local name	Use Index (UI) (%)	Use value (UV)	Treatment associations
1	*Osyris lanceolata, Hochst. & Steud.*	Olosesiai	68.9	0.03	Abdominal pains in children, Diarrhoea, Gonorrhoea
2	*Rhamnus staddo, A.Rich*	Olkokola	54.4	0.06	Strength/nutrient, supplement, sexually transmitted diseases, flu/cold
3	*Rapanea melanophloeos, (L.) Mez*	Seketet/ Lodwaa/ Oldwai	53.3	0.04	Fever, diarrhoea, anthelmintic and purgative
4	*Acacia nilotica, Schumach. and Thonn*	Olkiloriti/ Enkiloriti	52.2	0.09	Strengthened/ tonic, appetizer, body aches, stomachic, stamina,stimulant/excitant, antioxidant
5	*Zanthoxylum usambarense, (Engl.) Kokwaro*	Oloisuki/ Muguchwa	48.9	0.06	Malaria, backache, painful joints and rheumatism and also as an emetic and purgative.
6	*Rhamnus prinoides, L'Hér.*	Olkonyil/ Mukarakinga	45.6	0.04	Sexually transmitted infections, back and joint aches, arthritis, aids in digestion, tonic
7	*Warburgia ugandensis, Engl.*	Osokonoi/ Muthiga	41.1	0.10	Chest problems, pneumonia, diarrhoea, respiratory problems, stomach ache, malaria, STDs, malaria, abdominal disorders, arthritis
8	*Pappea capensis, Eckl. & Zeyh.*	Entimigomi/ Oltimigomi	36.7	0.07	Strengthens, food, fertility, stomach ache, stamina, aphrodisiac

S. No.	Scientific Name	Local name	Use Index (UI) (%)	Use value (UV)	Treatment associations
9	*Croton somalensis, Vatke and Pax ex Pax*	Enchani emburkel / Olchani olpurkel	34.4	0.02	Food, stomach ache
10	*Carissa edulis, Forssk.*	Olamuniaki/ Mukawa	32.2	0.06	Joint and muscle pain, gonorrhoea, chest pains, polio symptoms
11	*Toddalia asiatica, (L.) Lam.*	Ole parmunyo/ Mururue	31.1	0.03	Cold, fever and malaria,
12	*Embelia schimperi, Vatke*	Olchani onyokie/ enchani enkashe/ Onchani empuken	27.8	0.02	Anthelmintic, diarrhoea
13	*Azadirachta indica, A.Juss.*	Neem/ mwarobaini/ murubaini	24.4	0.04	Malaria, fever, aches, pains, skin infections
14	*Turraea abyssinica, Hochst.*	Enchani enkashe	22.2	0.01	Anthelmintic
15	*Euclea divinorum, Hiern*	Olkinyei/ Osojo/ Mukinyai	21.1	0.11	Ulcers, diarrhoea, wounds, arthritis, snakebites, headache, toothache, gonorrhoea, purgative (in soup) and as a tonic for anaemia,
16	*Plumbago zeylanica, L.*	Enekuseron/Enchani enkusero	21.1	0.03	Skin diseases, infections and intestinal worms
17	*Albizia anthelmintica, Brongn.*	Emugutan	20.0	0.06	Purgative, tonic, de-wormer, fever and malaria
18	*Clerodendrum myricoides, (Hochst.) R.Br. ex Vatke*	Olmakutukut	20.0	0.12	Gonorrhoea, rabies, measles, TB, colic, eye disease, malaria, swellings in the body, wound dressings, asthma and as aphrodisiac
19	*Olea europaea, L.*	Oloirien / Mutamaiyu	20.0	0.02	Malaria, pneumonia
20	*Strychnos henningsii, Gilg*	Entipilikwa/ Oltipilikwa/ Mteta	18.9	0.03	Rheumatism, gastrointestinal complaints and snake bites

S. No.	Scientific Name	Local name	Use Index (UI) (%)	Use value (UV)	Treatment associations
21	*Rhoicissus tridentate, (L.f.) Wild and R.B.Drumm.*	Orarait/ Olkilenyai/ Ole rubat	17.8	0.06	infertility, stomach, kidney and bladder complaints, dysmenorrhea
22	*Albizia amara, Boiv.*	Olpere longo	16.7	0.06	Piles, diarrhoea and gonorrhoea, cough, ulcers, dandruff and malaria, Cancer
23	*Ximenia americana, L.*	Enkamai/ Olamai	16.7	0.06	Stomach-ache in kids, food, tonic, constipation, HIV
24	*Croton dichogamus, Pax*	Olokirdingai/ Enkitarru	15.6	0.03	Polio-like symptoms, gonorrhoea, chest pain
25	*Periploca linearifolia, Quart.-Dill. & A.Rich.*	Osinandei	14.4	0.01	Dental hygiene
26	*Salvadora persica, L*	Oremit/ Eremit	14.4	0.08	Eye infections, worms, malaria, stomach ache, constipation, cold, teeth hygiene, respiratory infections
27	*Combretum molle, R.Br. ex G.Don*	Olmaroroi	13.3	0.12	Abdominal pain, colic, constipation, intestinal worms and dysentery, fever, malaria, headache, backache, HIV infections, cough
28	*Trimeria grandifolia, (Hochst.) Warb.*	Oledat/ muhinduhindu	13.3	0.02	Soup, Malaria
29	*Vernonia brachycalyx, O.Hoffm.*	Ologumati	13.3	0.01	Malaria
30	*Urtica massaica, Mildbr.*	Entamejoi /Ndamesoi/ Thabai	11.1	0.04	Malaria, fractures and venereal diseases, stomach-ache
31	*Piliostigma thonningii, (Schum.) Milne-Redh.*	Orbukoi/ Olbukoi	10.0	0.08	Wounds and ulcers as a haemostatic and to promote healing, diuretic, diarrhoea,

S. No.	Scientific Name	Local name	Use Index (UI) (%)	Use value (UV)	Treatment associations
					dysentery, worms and other intestinal problems
32	*Acokanthera schimperi, (A.DC.) Benth. & Hook.f. ex Schweinf.*	Olmorijoi	8.9	0.01	Malaria
33	*Prunus Africana, (Hook.fil.) Kalkm*	Olkoijuk / Muiri	8.9	0.02	Cancer, stomach-ache
34	*Searsia natalensis, (Bernh. ex C.Krauss) F.A.Barkley*	Olmisigiyioi/Muthigiu	8.9	0.10	Worm infections, chest problems, pneumonia, respiratory disorders, stomachic, malaria, digestive disorders, gonorrhea
35	*Turraea mombassana, C. DC.*	Enchani enkashe	8.9	0.03	Excess bile, malaria and other fevers.
36	*Aloe sp*	Osuguroi/ Esuguroi	6.7	0.01	Malaria
37	*Mystroxylon aethiopicum, (Thunb.) Loes*	Olodo nganayioi	6.7	0.01	Colic pain, especially in children
38	*Psiadia punctulata, Vatke*	Olabaai/ Olabaai la partolu	6.7	0.03	colds, headache and abdominal pains
39	*Myrica salicifolia, Hochst. ex A.Rich.*	Olkitoloswa	5.6	0.02	Tonsillitis, throat infections
40	*Acacia mellifera, Vahl.*	Oiti	4.4	0.04	Postpartum tonic, appetizer, sore throat, stomach ache
41	*Acacia nubica, Benth.*	Oldepe	4.4	0.04	Sexually transmitted infections, postpartum tonic, facilitate lactation, rejuvenation

S. No.	Scientific Name	Local name	Use Index (UI) (%)	Use value (UV)	Treatment associations
42	*Grewia bicolor, Juss*	Emporokwai / Esiteti	4.4	0.03	Eye infections, respiratory disorders, snake bite
43	*Maytenus senegalensis, (Lam.) Exell*	Olaimurunyai	4.4	0.10	Infectious diseases, arthritis, diarrhoea, dysentery, gastrointestinal diseases, menstrual pain, eye infections, nausea, , severe headache, aphrodisiac
44	*Ajuga remota, Benth*	Osarara	3.3	0.02	Malaria, fever, infections

4.4 Identification of plant species commonly traded as herbal products using DNA barcoding

When applying DNA techniques to identify the commonly traded herbal products in the study counties, the study realized that the samples or herbal products were from single plant species or from mixed species which had been mixed but traded as one. This section reports findings of DNA analysis of both mixed species herbal products and single species herbal products as sampled in the study area.

4.4.1 Mixed species herbal products identification

A total of 72 mixed species herbal products from the three counties were analyzed. Ten out of the 72 samples did not yield quantifiable DNA using spectro-biophotometer at 260/ 280 ratios and on agarose gel electrophoresis and were therefore excluded from further analysis. Fifteen out of the 62 samples did not produce MOTUs for at least two of the replicates hence excluded from the data analysis. The remaining 47 DNA samples had a total of 257 species entries recorded that resulted to 73 species as listed on the labels when duplicates were excluded (Appendix IV). At family levels, the 73 species belong to 43 plant families. The 47 analysed samples had between two and thirteen species listed on the label with a mean of at least 5 species per sample (Table 4.5 and Appendix V).

Table 4.5: Mixed species samples analyzed using metabarcoding (pages 59 to 61, extracted from appendix V)

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
10	MAN13	3	*Rumex crispus* L. (Yellow dock) *Salvadora persica* L. *Azadirachta indica* A.Juss.	*Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Balanites glabra* Mildbr. & Schltr. *Ipomoea batatas* (L.) Lam. *Salvadora persica* L.	No species on label was detected
18	MAN27	4	*Albizia anthelmintica* Brongn *Croton dichogamus* Pax *Psiadia punctulata* (DC.) Vatke *Warburgia ugandensis* Sprague	*Albizia anthelmintica* Brongn *Croton dichogamus* Pax *Warburgia ugandensis* Sprague *Ajuga remota* Benth. *Cymbopogon citratus* (DC.) Stapf *Triticum aestivum* L. *Triticum turgidum* L. *Zea mays* L.	Only 3 species on label were detected
21	MAN30	8	*Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Euclea divinorum* Hiern *Myrsine africana* L. *Osyris lanceolata* Hochst. & Steud. *Rhamnus prinoides* L'Hér.	*Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Euclea divinorum* Hiern *Myrsine africana* L. *Osyris lanceolata* Hochst. & Steud. *Rhamnus prinoides* L'Hér.	Only 7 species on label detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Withania somnifera (L.) Dunal *Zanthoxylum usambarense* (Engl.) Kokwaro	*Withania somnifera* (L.) Dunal *Zanthoxylum sp*. *Ajuga remota* Benth. *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Carica papaya* L. *Ocimum gratissimum* L. *Pittosporum mannii* Hook.f. *Senna italica* Mill. *Zea mays* L.	
24	MAN33	5	*Albizia anthelmintica* Brongn *Amaranthus dubius* Mart. *Azadirachta indica* A.Juss. *Moringa oleifera* Lam. *Osyris lanceolata* Hochst. & Steud.	*Amaranthus dubius* Mart. *Azadirachta indica* A.Juss. *Moringa oleifera* Lam. *Osyris lanceolata* Hochst. & Steud. *Aloe vera* (L.) Burm.f. *Oryza sativa* L. *Persea americana* Mill. *Piper sp*. *Pittosporum mannii* Hook.f. *Salvia officinalis* L.	All species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
				Rosmarinus officinalis L *Senna occidentalis* (L.) Link *Triticum turgidum* L. *Urtica dioica* L. *Warburgia ugandensis* Sprague *Zea mays* L.	
33	MAN46	4	*Hibiscus flavifolius* Ulbr.(Hibiscus) Mentha (mint) *Rosmarinus officinalis* L (rosemary) *Urtica dioica* L. (stinging neetle)	*Urtica dioica* L. *Urtica massaica* Mildbr.	Only 1 species on label detected
35	MAN48	3	Stry*chnos henningsii* Gilg *Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f.	*Strychnos sp.* *Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Carica papaya* L.	Only 2 species on label detected

4.4.2 Identification of mixed species herbal samples using metabarcoding

From the next generation sequencing of the 47 samples using nrITS2 marker, a total of 492 blast hits produced by at least 2 replicates as per selection criteria were recorded resulting to one hundred and four species entries (Table 4.6). Out of the 104 entries, 96 were identified to species level, 4 to genus level and 4 to family level. The minimum prediction accuracy used was 99 %. The minimum read length was 150 bp with a maximum 400 bp. The occurrence of each detected species in relation to the 47 samples tested is in Table 4.6. Eleven species with at least 10 occurrences listed from the highest were *Albizia anthelmintica* Brongn (60 %), *Urtica dioica* L. (47 %), *Pittosporum mannii* Hook.f. (*Pittosporum viridiflorum* Sims) (38 %), *Warburgia ugandensis* Sprague (38 %), *Osyris lanceolata*. Hochst. & Steud (36 %), *Triticum aestivum* L. (36 %), *Aloe vera* (L.) Burm.f. (32 %), *Salvadora persica* L. (28 %), *Ajuga remota* Benth. (*Ajuga integrifolia* Buch.-Ham.) (26 %), *Amaranthus dubius* Mart. ex Thell (21 %) and *Senna italica* Mill. (21 %). Maize (*Zea mays* L.) was the most detected filler at 66 % occurrence followed by *Triticum aestivum* L. (36 %) and *Oryza sativa* L. (23 %). The results of species detected from the mixture species samples are as summarised in Table 4.6 and Appendix V

Table 4.6: Species identified in mixed herbal products by DNA metabarcoding technology (pages 63 to 67)

S. No	Plant Species identified	Genus	Family	Mixed samples occurrence (N = 47)	Percentage (%) occurrence
1	*Zea mays* L.	*Zea*	Poaceae	31	66
2	*Albizia anthelmintica* Brongn	*Albizia*	Fabaceae	28	60
3	*Urtica dioica* L.	*Urtica*	Urticaceae	22	47
4	*Pittosporum mannii* Hook.f. (*Pittosporum viridiflorum* Sims)	*Pittosporum*	Pittosporaceae	18	38
5	*Warburgia ugandensis* Sprague	*Warburgia*	Canellaceae	18	38
6	*Osyris lanceolata* Hochst. & Steud.	*Osyris*	Santalaceae	17	36
7	*Triticum aestivum* L.	*Triticum*	Poaceae	17	36
8	*Aloe vera* (L.) Burm.f.	*Aloe*	Xanthorrhoeaceae	15	32
9	*Salvadora persica* L.	*Salvadora*	Salvadoraceae	13	28
10	*Ajuga remota* Benth. (*Ajuga integrifolia* Buch.-Ham.)	*Ajuga*	Lamiaceae	12	26
11	*Oryza sativa* L.	*Oryza*	Poaceae	11	23
12	*Amaranthus dubius* Mart. ex Thell.	*Amaranthus*	Amaranthaceae	10	21
13	*Senna italica* Mill.	*Senna*	Fabaceae	10	21
14	*Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord."	*Baccharoides*	Compositae	9	19
15	*Ocimum gratissimum* L.	*Ocimum*	Lamiaceae	9	19
16	*Pennisetum glaucum* (L.) R.Br.	*Pennisetum*	Poaceae	8	17

S. No	Plant Species identified	Genus	Family	Mixed samples occurrence (N = 47)	Percentage (%) occurrence
17	*Croton dichogamus* Pax	*Croton*	Euphorbiaceae	7	15
18	*Dombeya rotundifolia* (Hochst.) Planch.	*Dombeya*	Malvaceae	7	15
19	*Moringa oleifera* Lam.	*Moringa*	Moringaceae	7	15
20	*Rosmarinus officinalis* L.	*Rosmarinus*	Lamiaceae	7	15
21	*Sorghum bicolor* (L.) Moench	*Sorghum*	Poaceae	7	15
22	*Azadirachta indica* A.Juss.	*Azadirachta*	Meliaceae	6	13
23	*Balanites glabra* Mildbr. and Schltr.	*Balanites*	Zygophyllaceae	6	13
24	*Carica papaya* L.	*Carica*	Caricaceae	6	13
25	*Euclea divinorum* Hiern	*Euclea*	Ebenaceae	6	13
26	*Rhamnus prinoides* L'Hér.	*Rhamnus*	Rhamnaceae	6	13
27	*Zanthoxylum usambarense* (Engl.) Kokwaro	*Zanthoxylum*	Rutaceae	6	13
28	*Clerodendrum heterophyllum* (Poir.) R.Br. (*Volkameria heterophylla* Poir.)	*Clerodendrum*	Lamiaceae	5	11
29	*Faurea saligna* Harv.	*Faurea*	Proteaceae	5	11
30	*Ocimum basilicum* L.	*Ocimum*	Lamiaceae	5	11
31	*Rotheca myricoides* (Hochst.) Steane & Mabb.	*Rotheca*	Lamiaceae	5	11
32	*Secale cereale* L.	*Secale*	Poaceae	5	11
33	*Senna occidentalis* (L.) Link	*Senna*	Fabaceae	5	11
34	*Sesamum indicum* L.	*Sesamum*	Pedaliaceae	5	11
35	*Withania somnifera* (L.) Dunal	*Withania*	Solanaceae	5	11
36	*Carissa edulis* Forssk. (*Carissa spinarum* L.)	*Carissa*	Apocynaceae	4	9
37	*Croton megalocarpus* Hutch	*Croton*	Euphorbiaceae	4	9
38	*Galinsoga parviflora* Cav.	*Galinsoga*	Compositae	4	9

S. No	Plant Species identified	Genus	Family	Mixed samples occurrence (N = 47)	Percentage (%) occurrence
39	*Gynandropsis gynandra* (L.) Briq. (*Cleome gynandra* L.)	*Gynandropsis*	Cleomaceae	4	9
40	*Hordeum vulgare* L.	*Hordeum*	Poaceae	4	9
41	*Ipomoea batatas* (L.) Lam.	*Ipomoea*	Convolvulaceae	4	9
42	*Rumex crispus* L.	*Rumex*	Polygonaceae	4	9
43	*Bidens pilosa* L.	*Bidens*	Compositae	3	6
44	*Capsicum annuum* L.	*Capsicum*	Solanaceae	3	6
45	*Cuminum cyminum* L.	*Cuminum*	Apiaceae	3	6
46	*Cymbopogon citratus* (DC.) Stapf	*Cymbopogon*	Poaceae	3	6
47	*Cynodon aethiopicus* Clayton & Harlan	*Cynodon*	Poaceae	3	6
48	*Musa acuminata* Colla	*Musa*	Musaceae	3	6
49	*Olea europaea* L.	*Olea*	Oleaceae	3	6
50	*Pappea capensis* Eckl. & Zeyh.	*Pappea*	Sapindaceae	3	6
51	*Rhamnus staddo* A.Rich.	*Rhamnus*	Rhamnaceae	3	6
52	*Solanum lycopersicum* L.	*Solanum*	Solanaceae	3	6
53	*Urtica massaica* Mildbr.	*Urtica*	Urticaceae	3	6
54	*Acacia kirkii* Oliv.	*Acacia*	Fabaceae	2	4
55	*Clausena anisata* (Willd.) Hook.f. ex Benth.	*Clausena*	Rutaceae	2	4
56	*Coriandrum sativum* L.	*Coriandrum*	Apiaceae	2	4
57	*Cucurbita pepo* L.	*Cucurbita*	Cucurbitaceae	2	4
58	*Cynodon dactylon* (L.) Pers.	*Cynodon*	Poaceae	2	4
59	*Lantana camara* L.	*Lantana*	Verbenaceae	2	4
60	*Myrsine africana* L.	*Myrsine*	Primulaceae	2	4
61	*Nicotiana tabacum* L.	*Nicotiana*	Solanaceae	2	4

S. No	Plant Species identified	Genus	Family	Mixed samples occurrence (N = 47)	Percentage (%) occurrence
62	*Persea americana* Mill.	*Persea*	Lauraceae	2	4
63	*Solanum melongena* L.	*Solanum*	Solanaceae	2	4
64	*Solanum nigrum* L.	*Solanum*	Solanaceae	2	4
65	*Solanum tuberosum* L.	*Solanum*	Solanaceae	2	4
66	*Trigonella foenum-graecum* L	*Trigonella*	Fabaceae	2	4
67	*Vernonia brachycalyx* O.Hoffm.	*Vernonia*	Compositae	2	4
68	*Aloe secundiflora* Engl.	*Aloe*	Xanthorrhoeaceae	1	2
69	*Avena sativa* L.	*Avena*	Poaceae	1	2
70	Cassia sp. (*Cassia abbreviata*)	*Cassia*	Fabaceae	1	2
71	*Cissus rotundifolia* Vahl	*Cissus*	Vitaceae	1	2
72	*Cordia monoica* Roxb.	*Cordia*	Boraginaceae	1	2
73	*Dactyloctenium aegyptium* (L.) Willd.	*Dactyloctenium*	Poaceae	1	2
74	*Delonix elata* (L.) Gamble	*Delonix*	Fabaceae	1	2
75	*Dodonaea viscosa* (L.) Jacq.	*Dodonaea*	Sapindaceae	1	2
76	*Duranta erecta* L.	*Duranta*	Verbenaceae	1	2
77	*Grevillea robusta* A.Cunn. ex R.Br.	*Grevillea*	Proteaceae	1	2
78	*Grewia similis* K.Schum	*Grewia*	Malvaceae	1	2
79	*Jatropha curcas* L.	*Jatropha*	Euphorbiaceae	1	2
80	*Leucaena leucocephala* (Lam.) de Wit	*Leucaena*	Fabaceae	1	2
81	*Myristica fragrans* Houtt.	*Myristica*	Myristicaceae	1	2
82	*Oryza barthii* A.Chev.	*Oryza*	Poaceae	1	2
83	*Pentas micrantha* Baker	*Pentas*	Rubiaceae	1	2
84	Piper sp.	*Piper*	Piperaceae	1	2

S. No	Plant Species identified	Genus	Family	Mixed samples occurrence (N = 47)	Percentage (%) occurrence
85	*Saccharum officinarum* L.	*Saccharum*	Poaceae	1	2
86	*Salvia officinalis* L.	*Salvia*	Lamiaceae	1	2
87	*Salvia hispanica* L. (*Salvia officinalis* L.)	*Salvia*	Lamiaceae	1	2
88	*Schinus molle* L. (Annonaceae sp.)	*Schinus*	Anacardiaceae	1	2
89	*Stellaria media* (L.) Vill.	*Stellaria*	Caryophyllaceae	1	2
90	*Strychnos henningsii* Gilg	*Strychnos*	Loganiaceae	1	2
91	Strychnos sp.	*Strychnos*	Loganiaceae	1	2
92	*Teclea simplicifolia* (Engl.) I. Verd. (*Vepris simplicifolia* (Engl.) Mziray)	*Vepris*	Rutaceae	1	2
93	*Thunbergia alata* Bojer ex Sims	*Thunbergia*	Acanthaceae	1	2
94	*Vachellia drepanolobium* (Harms ex Sjostedt) P.J.H. Hurter	*Vachellia*	Fabaceae	1	2
95	*Vangueria infausta* Burch.	*Vangueria*	Rubiaceae	1	2
96	*Ximenia americana* L.	*Ximenia*	Olacaceae	1	2
97	*Zanthoxylum chalybeum* Engl.	*Zanthoxylum*	Rutaceae	1	2
98	Zanthoxylum sp.	*Zanthoxylum*	Rutaceae	3	6
99	*Zehneria scabra* Sond.	*Zehneria*	Cucurbitaceae	1	2
100	Zehneria sp	*Zehneria*	Cucurbitaceae	2	4
101	Rutaceae sp.		Rutaceae	3	6
102	Cucurbitaceae sp.		Cucurbitaceae	2	4
103	Anacardiaceae sp.		Anacardiaceae	2	4
104	Annonaceae sp.		Annonaceae	1	2

4.4.3 Identification of single species herbal samples using DNA barcoding

Forty four single species samples common across the three counties that were analyzed had DNA concentrations ranging from 0.01ng to 38ng and 260/280nm ranging from 0.1 to 1.6. In Kajiado, out of the 44 DNA samples, three had low concentrations that did not produce any amplicon. The remaining 41 samples were amplified successfully. Five amplicons produced very low quality sequences that could not be assembled or edited for further analysis. The 3 samples that did not produce amplicons and the five samples that produced low quality sequences were excluded in the final analysis.

In Nairobi, only five out of the 44 DNA samples had low concentrations. The remaining 39 samples were amplified successfully although 3 of them did not produce quality sequences for further analysis. The five samples with poor quality DNA and the 3 that produced low quality sequences were excluded from the final analysis.

In Narok only four out of the 44 DNA samples had low concentrations. The remaining 40 samples were amplified successfully even though 3 amplicons did not produce quality DNA. The four samples with low DNA concentrations together with the 3 samples that produced low quality sequence were excluded in the final analysis.

Out of the 44 samples analyzed, the technology identified 36 from Nairobi and Kajiado and 37 from Narok to species level respectively as shown in Table 4.7. Success of amplification was confirmed in 1.5 % agarose gel against a 100 bp DNA ladder (Plate 4.1). The *ITS* marker identified most of the samples to species level, followed by *rbcL* and *matK* had least amplification and identification success.

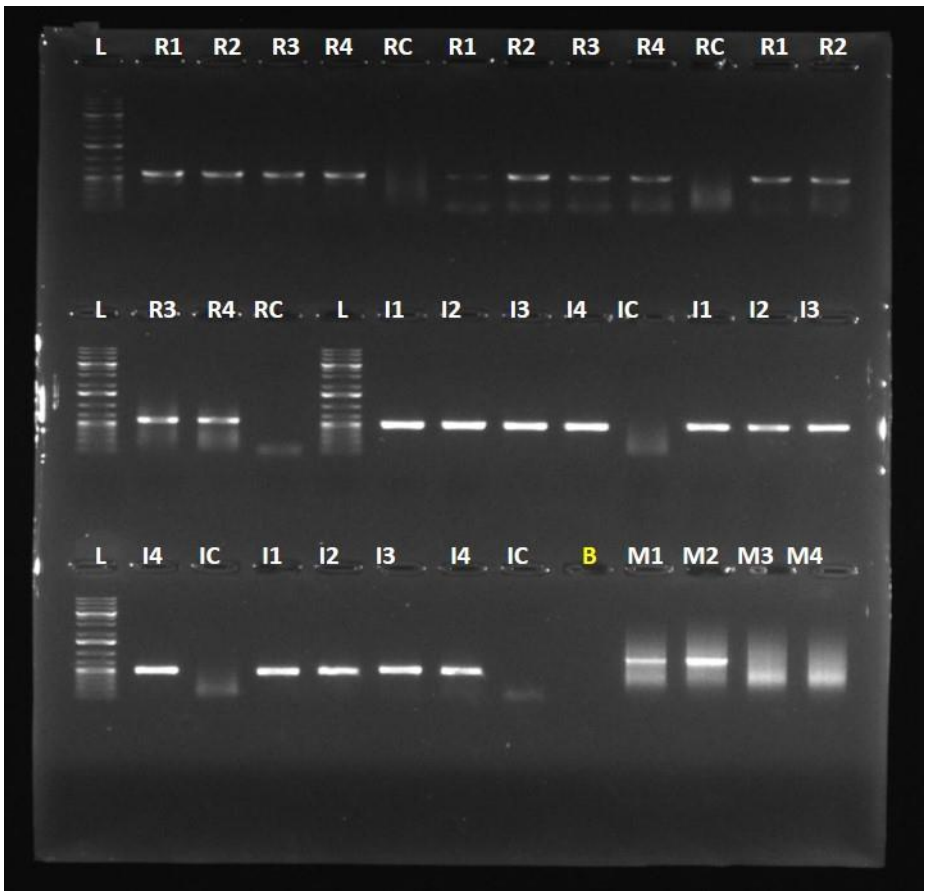

Plate 4.1: Sample gel photo for single species herbal products amplified with rbcL (R), ITS (I) and matK (M) markers.

Key: L, 100 bp marker (Magbio), R1-R4, *Albizia antihelmintica, Aloe vera*, *Albizia amara and Carrissa edulis* DNA samples replicates for rbcL; I1-I4, *Albizia antihelmintica*, *Aloe vera*, *Albizia amara and Carrissa edulis* DNA samples replicates for ITS; M1-M4, *Albizia antihelmintica, Aloe vera*, *Albizia amara and Carrissa edulis* DNA samples replicate 1 for matk; RC and IC, negative controls; B, blank.

Table 4.7: Summary results of DNA identification of single species herbal product samples using ITS, rbcL and matK markers (pages 70 to 73)

			Kajiado			Nairobi			Narok		
S. No	**Species name on label**	**Plant Family**	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***
1	*Acacia mellifera*, Vahl.	Fabaceae	NS	NS	NS	1	0	0	*	*	*
2	*Acacia nilotica*, Schumach. & Thonn	Fabaceae	0	1	0	0	1	0	0	1	0
3	*Acacia nubica*, Benth.	Fabaceae	0	*Acacia nilotica*	0	*Urtica dioca*	0	0	0	*Acacia nilotica*	
4	*Acokanthera schimperi* (A.DC.) Schweinf.	Apocynaceae	1	0	0	*Urtica dioca*	0	0	*Acacia nilotica*	0	0
5	*Ajuga remota*, Benth - (Ajuga sp)	Lamiaceae	*	*	*	1	0	1	1	1	1
6	*Albizia amara*, Boiv.	Fabaceae	1	1	0	1	1	0	1	1	0
7	*Albizia anthelmintica*, Brongn.	Fabaceae	1	0	1	1	0	1	1	0	1
8	Aloe sp (*Aloe vera*)	Aloeceae	1		1	1		1	1		1
9	*Azadirachta indica*, A.Juss.	Meliaceae	1	1	0	*Melia azedarach*	1	0	1	1	0
10	*Carissa edulis*, Forssk. (*Carissa spinarum* L.)	Apocynaceae	1	1	0	1	1	0	1	1	0
11	*Clerodendrum myricoides*, (Hochst.) R.Br. ex Vatke (*Rotheca*	Verbenaceae	1	1	0	1	1	0	1	1	0

			Kajiado			Nairobi			Narok		
S. No	**Species name on label**	**Plant Family**	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***
	myricoides (Hochst.) Steane & Mabb.)										
12	*Combretum molle*, R.Br. ex G.Don	Compretacaea e	1	1	0	1	1	0	1	1	0
13	*Croton dichogamus*, Pax	Euphorbiacea e	1	1	0	1	1	0	1	1	0
14	*Croton somalensis*, Vatke& Pax ex Pax	Euphorbiacea e	1			1			*Croton dichogam us*	1	1
15	*Embelia schimperi*, Vatke	Primulaceae	NS			*	*	*	1	B	0
16	*Euclea divinorum*, Hiern	Ebeneceae	1	1	0	1	1	0	1	1	0
17	*Grewia bicolor*, Juss	Malvaceae	1	1	0	1	1	0	1	1	0
18	*Maytenus senegalensis*, (Lam.) Exell	Celastraceae	*	*	*	NS	NS	NS	NS	NS	NS
19	*Myrica salicifolia*, Hochst. ex A.Rich.	Myricaceae	NS	NS	NS	*	*	*	*	*	*
20	*Mystroxylon aethiopicum*, (Thunb.) Loes	Celastraceae	NS	NS	NS	*	*	*	*	*	*
21	*Olea europaea*, L.	Oleaceae	1	1	0	1	1	0	1	1	0
22	*Osyris lanceolata*, Hochst. & Steud.	Santalaceae	1	1	0	1	1	0	1	1	0
23	*Pappea capensis*, Eckl. & Zeyh.	Sapindaceae	1	1	0	1	1	0	1	1	0

			Kajiado			Nairobi			Narok		
S. No	**Species name on label**	**Plant Family**	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***
24	*Periploca linearifolia*, Quart.-Dill. & A.Rich.	Apocynaceae	1	1	0	1	1	0	1	1	0
25	*Piliostigma thonningii*, (Schum.) Milne-Redh.	Fabaceae	1	1	0	NS	NS	NS	1	1	0
26	*Plumbago zeylanica*, L.	Plumbaginacea	0	1	0	*	*	*	NS	NS	NS
27	*Prunus Africana*, (Hook.fil.) Kalkm	Rosaceae	*Osyris lanceolata*	*Osyris lanceolata*	0	*Urtica dioca*	*Urtica dioca*	0	*Osyris lanceolata*	*Osyris lanceolata*	0
28	*Psiadia punctulata*, Vatke	Asteraceae	*	*	*	1			1		
29	*Rapanea melanophloeos*, (L.) Mez	Mimosaceae	1	1		1	1		1	1	
30	*Rhamnus prinoides*, L'Hér.	Rhamnaceae	1	1		1 *Rhamnus staddo*	1 *Rhamnus staddo*		1	1	
31	*Rhamnus staddo*, A.Rich	Rhamnaceae	1	1		1	1		1	1	
32	*Rhoicissus tridentata*, (L.f.) Wild & R.B.Drumm.	Vitaceae	0	1	0	*Croton dichogamus*	*Croton dichogamus*	0	0	*Urtica dioca*	0
33	*Salvadora persica*, L	Salvadoraceae	1	1		1	1		1	1	

			Kajiado			Nairobi			Narok		
S. No	**Species name on label**	**Plant Family**	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***
34	*Searsia natalensis*, (Bernh. ex C.Krauss) F.A.Barkley	Anacardiaceae	1	1	0	1	0	0	1	1	0
35	*Strychnos henningsii*, Gilg	Loganiaceae	1	1		1	1		1	1	
36	*Toddalia asiatica*, (L.) Lam.	Rutaceae	1	1		*Urtica dioca*	*Urtica dioca*		1	1	
37	*Trimeria grandifolia*, (Hochst.) Warb.	Salicaceae	NS	NS	NS	NS	NS	NS	*	*	*
38	*Turraea abyssinica*, Hochst.	Meliaceae	*Turraea mombassana*	0	0	*Turraea mombassana*	*Turraea mombassana*	0	1	1	0
39	*Turraea mombassana*, C. DC.	Meliaceae	1	1	0	1	0	0	1	1	0
40	*Urtica massaica*, Mildbr.	Urticaceae	1	1	1	*Urtica dioca*	*Urtica dioca*	0	1	1	1
41	*Vernonia brachycalyx*, O.Hoffm.	Compositae	1	1	0	1	1	0	1	1	0
42	*Warburgia ugandensis*, Engl.	Canellaceae	1	1	1	1	1	0	1	1	0
43	*Ximenia americana*, L.	Olacaceae	1	0	0	*	*	*	1	0	0
44	*Zanthoxylum usambarense*, (Engl.) Kokwaro	Rutaceae	1	0	0	*Warbugia ugandensis*	0	0	NS	NS	NS

			Kajiado			Nairobi			Narok		
S. No	**Species name on label**	**Plant Family**	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***	***ITS***	***rbcL***	***mat K***
	Totals		**30**	**27**	**4**	**24**	**19**	**3**	**31**	**28**	**5**

Key: NS, represents samples that did not produce quality sequences that could be edited for blasting; * Represents samples with low DNA concentrations-no amplicons, I indicate identified as listed, 0 no species identified.

4.5 Generation of reference barcodes of commonly traded herbal plants using DNA technology

Based on market survey data gathered, 44 plant species were traded across the three study regions whose voucher specimens were deposited at East African herbarium at National Museums of Kenya. The DNA extracted from their corresponding leaf specimens had concentration ranging from 23.4 to 120.1 µg/µL. The partial *ITS2*, *rbcL* and *matK* consensus barcode sequences generated were deposited in the NCBI GenBank database with a 'barcode' tag. The reference barcodes are shown in Table 4.8 and an example of a barcode sequence MT231403 published in GenBank is in Table 4.9.

Table 4.8: Generated reference barcodes (pages 74 to 75)

Accession number	Accession number	Accession number	Accession number	Accession number
KU748252	MH630248	MN177150	MT137498	MT231424
KU748253	MH630249	MN177151	MT137499	MT231425
KU748257	MH630250	MN177152	MT137500	MT231426
KU748290	MH630251	MN177153	MT137501	MT231427
KY556640	MH630252	MN177154	MT137502	MT231428
MT137514	MH630253	MN177155	MT137503	MT231429
KU747989	MH630254	MN177156	MT137504	MT231430
KU747990	MH630255	MN177157	MT137505	MT231431
KU748064	MH630256	MN177158	MT137506	MT231432
KU748065	MH630257	MN177159	MT137507	MT231433
KU748066	MH630258	MN177160	MT137508	MT231434
KU748067	MH630259	MN177161	MT137509	MT231435
KU748068	MH630260	MN177162	MT137510	MT231436
KU748069	MH630261	MN177163	MT137511	MT231437
KU748070	MH630262	MN177164	MT137512	MT231438
KU748071	MK696137	MN177165	MT137513	MT231439
KU748072	MK696138	MN337105	MT137514	MT231440
KU748167	MK696139	MN337106	MT137515	MT231441
KU748168	MK696140	MN337107	MT231385	MT231442
KU748169	MK696141	MN337108	MT231386	MT231443

Accession number	Accession number	Accession number	Accession number	Accession number
KU748170	MK696142	MN337109	MT231387	MW567244
KU748171	MK696143	MN337110	MT231388	MW567245
KU748196	MK696144	MN337111	MT231389	MW567246
KU748197	MK696145	MN337112	MT231390	MW567247
KU748198	MK696146	MN337113	MT231391	MW567248
KU748199	MK696147	MN337114	MT231392	MW567249
KU748200	MK696148	MN337115	MT231393	MW567250
KU748218	MK696149	MN337116	MT231394	MW567251
KU748225	MK696150	MN337117	MT231395	MW567252
KU748226	MK696151	MN337118	MT231396	MW567253
KU748227	MK696152	MN337119	MT231397	MW567254
KU748228	MK696153	MN337120	MT231398	MW567255
KU748229	MK696154	MN337121	MT231399	MW567256
KU748230	MN177126	MN337122	MT231400	MW567257
KU748231	MN177127	MN337123	MT231401	MW567258
KU748232	MN177128	MN337124	MT231402	MW567259
KU748251	MN177129	MN337125	MT231403	MW567260
KU748252	MN177130	MN337126	MT231404	MW567261
KU748253	MN177131	MN337127	MT231405	MW567262
KU748254	MN177132	MN337128	MT231406	MW567263
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KU748256	MN177134	MT137482	MT231408	MW567265
KU748257	MN177135	MT137483	MT231409	MW567266
KU748258	MN177136	MT137484	MT231410	MW567267
KU748259	MN177137	MT137485	MT231411	MW567268
KU748260	MN177138	MT137486	MT231412	MW567269
KU748261	MN177139	MT137487	MT231413	MW567270
KU748272	MN177140	MT137488	MT231414	MW567271
KU748282	MN177141	MT137489	MT231415	MW567272
KU748284	MN177142	MT137490	MT231416	MW567273
KU748286	MN177143	MT137491	MT231417	MW567274
KU748330	MN177144	MT137492	MT231418	MW567275
KU748333	MN177145	MT137493	MT231419	MW567276
KU748339	MN177146	MT137494	MT231420	MW567277
KU748369	MN177147	MT137495	MT231421	MW567278
KU748375	MN177148	MT137496	MT231422	MW567279
KX377524	MN177149	MT137497	MT231423	MW567280
MW567281	MW567282	MW567283		

The actual sequences, sequencing primers, and other meta data for published sequences can be accessed through the link:

https://www.ncbi.nlm.nih.gov/nuccore/?term=Mwaura+A.

Table 4.9: Reference barcode sequence with accession number MT231403 as published in the GenBank, (https://www.ncbi.nlm.nih.gov/nuccore/?term=Mwaura+A.) (pages 76 to 77)

GenBank
Osyris lanceolata voucher NMK:EA 13476 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast

GenBank: MT231403.1
FASTA Graphics PopSet
Go to:

LOCUS MT231403 543 bp DNA linear PLN 03-MAR-2021
DEFINITION Osyris lanceolata voucher NMK:EA 13476 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast.
ACCESSION MT231403
VERSION MT231403.1
KEYWORDS .
SOURCE chloroplast Osyris lanceolata
ORGANISM Osyris lanceolata
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliopsida; eudicotyledons; Gunneridae; Pentapetalae; Santalales; Santalaceae; Osyris.
REFERENCE 1 (bases 1 to 543)
AUTHORS Mwaura,A.
TITLE Direct Submission
JOURNAL Submitted (21-MAR-2020) Centre for Biodiversity, National Museums of Kenya, Museum Hill Road, Nairobi 00100, Kenya
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
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/organelle="plastid:chloroplast"
/mol_type="genomic DNA"
/specimen_voucher="NMK:EA 13476"

/db_xref="taxon:23413"
/country="Kenya"
/collection_date="2018"
/collected_by="Mwaura A and Mbale M"
/identified_by="Mathias Mbale"
/PCR_primers="fwd_name: rbcla-f, fwd_seq:
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CDS <1..>543
/gene="rbcL"
/codon_start=1
/transl_table=11
/product="ribulose-1,5-bisphosphate
carboxylase/oxygenase large subunit"
/protein_id="QSD98533.1"

/translation="SVGFKAGVKDYKLTYYTPDYETKDTDILAAFRVTPQPGVPPEE
A

GAAVAAESSTGTWTTVWTDGLTSLDRYKGRCYHIEPVAGEENQYIAYVAY
PLDLFEEG

SVTNMFTSIVGNVFGFKALRALRLEDLRIPPAYSKTFQGPPHGIQVERDKLN
KYGRPL
LGCTIKPKLGLSAKNYGRAVY"
ORIGIN
1 agtgtgggat tcaaagcggg ggttaaagat tacaaattga cttattatac tcctgattat
61 gaaaccaaag atactgatat cttggcagca ttccgagtaa ctcctcaacc gggagttccg
121 cctgaggaag ccggggccgc ggtagctgct gaatcttcta ctggtacatg gacaactgtg
181 tggaccgatg gacttaccag ccttgatcgt tacaaaggac gatgctacca catcgaaccc
241 gttgctggag aagaaaatca atatattgct tatgtagctt acccccttaga ccttttgaa
301 gaaggttctg ttactaacat gtttacttcc attgtgggta atgtgtttgg gttcaaagcc
361 ctgcgtgctc taaggttgga ggatctgcga atccctcctg cttattctaa aacttttcaa
421 ggcccacctc atggcatcca agttgagaga gataaattga acaagtatgg ccgtccatta
481 ttgggatgta ctattaaacc caaattgggg ttatccgcta agaactatgg tagagcggtt
541 tat
//

4.6 Authentication of single species herbal plant products

In Kajiado County, out of the 36 analyzed samples, 92 % (33) were categorized as authentic (DNA identified the species as labeled on the product) while 8 % were substituted with either a species in the same genera or with another species altogether as shown in Figure 4.4. The three samples that were not authentic were: Sample no. 14 where *Turraea abyssinica*, was substituted with *Turraea mombassana*; sample no. 33 where *Prunus africana* was substituted with *Osyris lanceolata*; and sample no. 41 where *Acacia nubica* was substituted with *Acacia nilotica.*

Nairobi County had the lowest authentic samples at 72 % (26) out the analyzed 36 samples while 28 % were either substituted with a close relative or a different species entirely (Figure 4.4). The 10 substituted samples included: sample no. 5 where *Zanthoxylum usambarense*, was substituted with *Warbugia ugandensis unrelated sample* (Figure 4.5); sample no. 6 where *Rhamnus prinoides* was substituted with *Rhamnus stado,* sample no. 11. where *Toddalia asiatica*, was substituted with *Urtica dioca;* sample no. 13 where *Azadirachta indica,* was substituted with *Melia azedarach;* sample no. 14 where *Turraea abyssinica* was substituted with *Turraea mombassana*; sample no. 21 where *Rhoicissus tridentata*, was substituted with *Croton dichogamus*; sample no. 30 where *Urtica massaica*, was substituted with *Urtica dioca;* sample no. 32 where *Acokanthera schimperi* was substituted with *Urtica dioca;* sample no. 33 where *Prunus africana*, was substituted with *Urtica dioca;* and sample no. 41 where *Acacia nubica* was substituted with *Urtica dioca.*

Out of the analyzed 37 samples from Narok region, 86 % (32) were authentic as shown in Figure 4.4 while 14 % were substituted. The five substituted samples were: Sample

no. 9 where *Croton somalensis*, was substituted with *Croton dichogamus;* sample no. 21 where *Rhoicissus tridentata*, was substituted with *Urtica dioca*; sample no. 32 where *Acokanthera schimperi* was substituted with *Acacia nilotica*; sample no. 33 where *Prunus africana* was substituted with *Osyris lanceolata*; and sample no. 41 where *Acacia nubica* was substituted with *Acacia nilotica.*

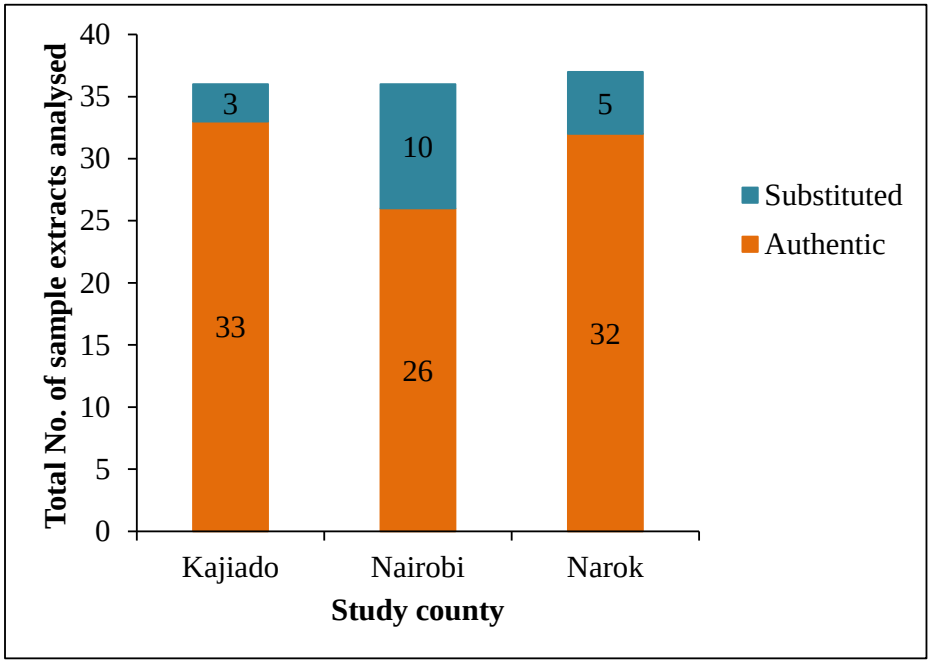

Figure 4.4: Herbal products authenticity for single plant species per study County.

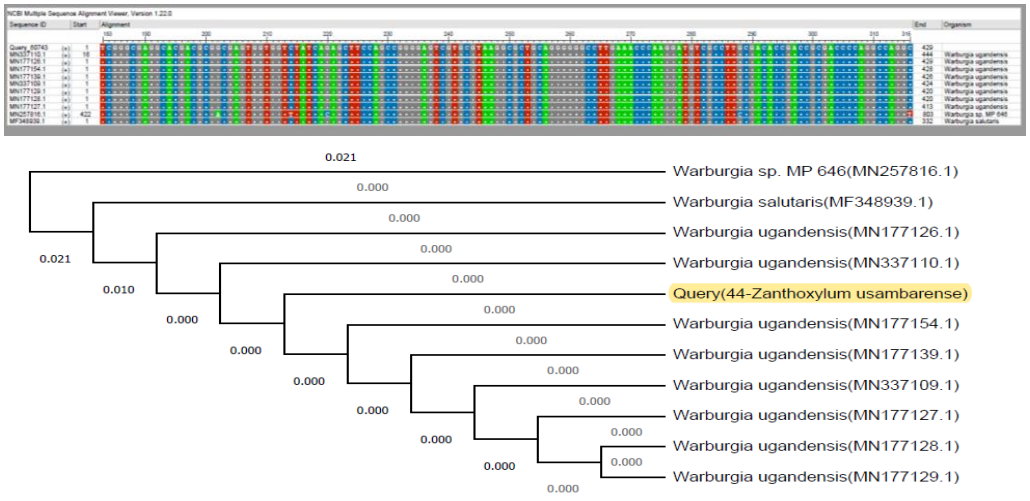

Figure 4.5: Multiple alignment and phylogenetic tree of *Zanthoxylum usambaranse* sample that was substituted with *Warbugia ugandensis.*

The comparative analysis of same species product across the three counties is shown in Table 4.10. Kajiado had the highest single plant species product samples corresponding to the species names as labeled in the market (91 % authentic herbal products), followed by Narok (88 %) while Nairobi had the lowest (69 %).

4.6.1 Identification of families based on common plant species

Among the families identified, Fabaceae had the most representation (13 %) from the confirmed samples followed by Apocynaceae and Meliaceae at 9 % each (Figure 4.6).

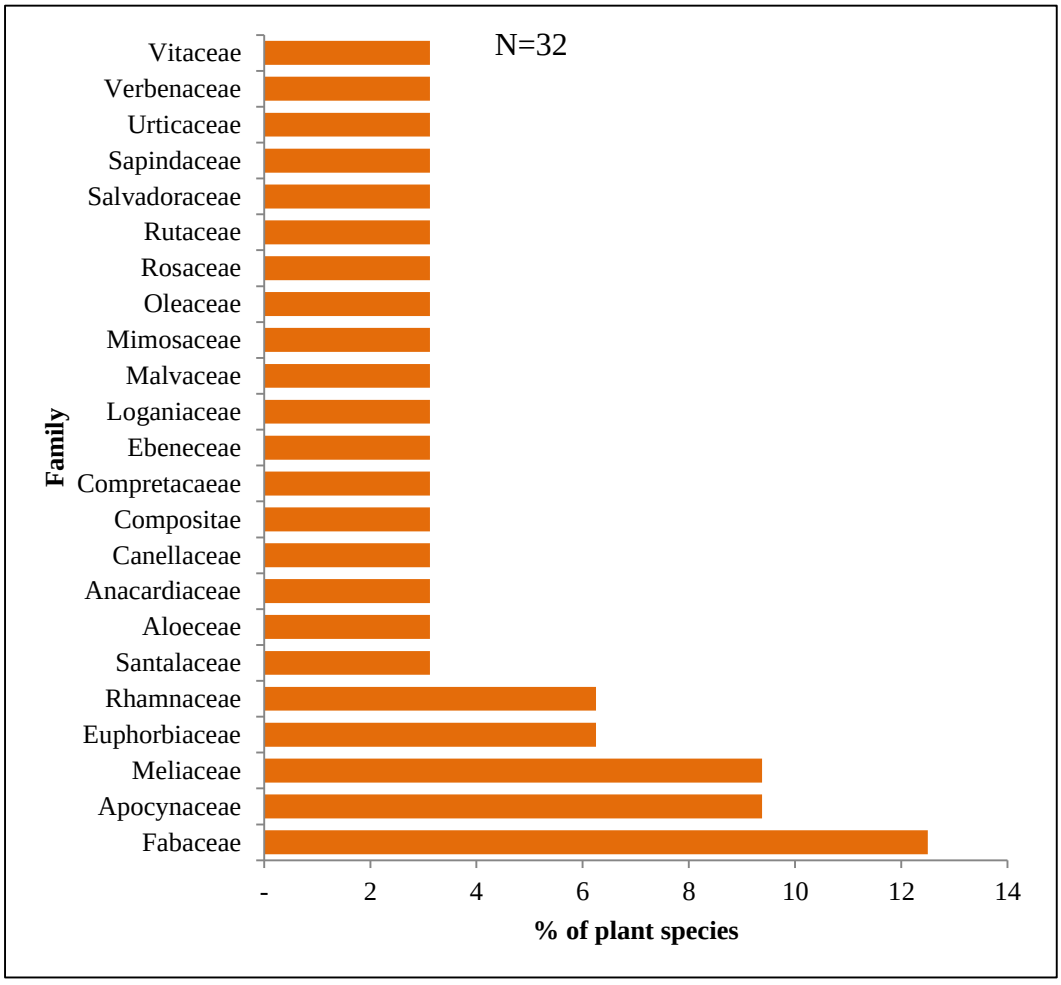

Figure 4.6: Percentage occurrence of plant families as identified by DNA barcoding in the single species products

Table 4.10: Comparative analysis of authenticity among the three study counties for single plant species product samples (pages 81 to 82)

S. No	Scientific Name	Family	Kajiado	Nairobi	Narok
1	*Osyris lanceolata*, Hochst. & Steud.	Santalaceae	1	1	1
2	*Rhamnus staddo*, A.Rich	Rhamnaceae	1	1	1
3	*Rapanea melanophloeos*, (L.) Mez	Mimosaceae	1	1	1
4	*Acacia nilotica*, Schumach. & Thonn	Fabaceae	1	1	1
6	*Rhamnus prinoides*, L'Hér.	Rhamnaceae	1	0	1
7	*Warburgia ugandensis*, Engl.	Canellaceae	1	1	1
8	*Pappea capensis*, Eckl. & Zeyh.	Sapindaceae	1	1	1
9	*Croton somalensis*, Vatke & Pax ex Pax	Euphorbiaceae	1	0	1
10	*Carissa edulis*, Forssk. (Carissa spinarum L.)	Apocynaceae	1	1	1
11	*Toddalia asiatica*, (L.) Lam.	Rutaceae	1	0	1
13	*Azadirachta indica*, A.Juss.	Meliaceae	1	0	1
14	*Turraea abyssinica*, Hochst.	Meliaceae	0	0	1
15	*Euclea divinorum*, Hiern	Ebeneceae	1	1	1
17	*Albizia anthelmintica*, Brongn.	Fabaceae	1	1	1
18	*Clerodendrum myricoides*, (Hochst.) R.Br. ex Vatke (*Rotheca myricoides* (Hochst.) Steane & Mabb.)	Verbenaceae	1	1	1
19	*Olea europaea*, L.	Oleaceae	1	1	1
20	*Strychnos henningsii*, Gilg	Loganiaceae	1	1	1
21	*Rhoicissus tridentata*, (L.f.) Wild & R.B.Drumm.	Vitaceae	1	0	0
22	*Albizia amara*, Boiv.	Fabaceae	1	1	1
24	*Croton dichogamus*, Pax	Euphorbiaceae	1	1	1

S. No	Scientific Name	Family	Kajiado	Nairobi	Narok
25	*Periploca linearifolia*, Quart.-Dill. & A.Rich.	Apocynaceae	1	1	1
26	*Salvadora persica*, L	Salvadoraceae	1	1	1
27	*Combretum molle*, R.Br. ex G.Don	Compretacaeae	1	1	1
29	*Vernonia brachycalyx*, O.Hoffm.	Compositae	1	1	1
30	*Urtica massaica*, Mildbr.	Urticaceae	1	0	1
32	*Acokanthera schimperi* (A.DC.) Schweinf.	Apocynaceae	1	0	0
33	*Prunus africana*, (Hook.fil.) Kalkm	Rosaceae	0	0	0
34	*Searsia natalensis*, (Bernh. ex C.Krauss) F.A.Barkley	Anacardiaceae	1	1	1
35	*Turraea mombassana*, C. DC.	Meliaceae	1	1	1
36	*Aloe spp* (Aloe vera)	Aloeceae	1	1	1
41	*Acacia nubica*, Benth.	Fabaceae	0	0	0
42	*Grewia bicolor*, Juss	Malvaceae	1	1	1
Total authentic			**29**	**22**	**28**
Total samples (N = 32)			**32**	**32**	**32**
Percentage authenticity			**91%**	**69%**	**88%**

Key: 1, Species identified; 0, No species identified.

4.6.2 Herbal products substitution

In single species samples, most of the species listed were substituted with closely related and or look alike species in adultered samples. For instance, *Rhamnus prinoides*, L. 'Hér. was substituted, with *Rhamnus staddo*, A.Rich; *Croton somalensis*, Vatke and Pax ex Pax with *Croton dichogamus*, Pax; Azadirachta indica, A.Juss. with *Melia azedarach* L.; *Turraea abyssinica*, Hochst. with *Turraea mombassana*, C. DC; *Urtica massaica*, Mildbr with *Urtica dioica* L.; *Acacia nubica*, Benth with *Acacia nilotica*, Schumach. & Thonn. In some other samples, listed species were substituted with other non-related species for instance *Toddalia asiatica*, (L.) Lam with *Urtica dioica* L., *Rhoicissus tridentata*, (L.f.) Wild & R.B. Drumm with *Croton dichogamus* Pax and *Urtica dioica* L.; *Acokanthera schimperi* (A.DC.) Schweinf with *Urtica dioica* L. and *Acacia nilotica* Schumach. & Thonn. and *Prunus africana*, (Hook.fil.) Kalkm with *Osyris lanceolata* Hochst. & Steud and *Acacia nilotica*, Schumach. & Thonn.

4.7 Authentication of mixed plant species in traded herbal products

The study collected 72 mixed species samples of which 10 did not yield quantifiable DNA even after re-extraction process and were therefore excluded from further analysis. The remaining 62 samples were analyzed in triplicate for PCR and NGS sequencing.

The quality of sequences generated from the 62 samples from the Ion Torrent runs are as summarised in Table 4.11. The highest input raw sequence was 1,211,928 resulting to 947,770 good quality sequences. The lowest sequence mean length was 217 while the highest was 355. Only 47 out of the analysed 62 samples produced Molecular

Taxonomic Units (MOTUs) for at least 2 replicates hence were considered for further analysis.

Table 4.11: Summary of generated sequences quality for the 62 samples amplified (pages 85 to 87)

Sample no	Input sequences			Good sequences (frequency)			Good sequences (%)			Good sequences mean length		
	1	2	3	1	2	3	1	2	3	1	2	3
MAN1		56	1		42	0		75	0		333.33	
MAN2	68,046	82,789	59,388	54,995	65,376	47,802	80.82	78.97	80.49	333.09	331.93	338.44
MAN3		42			29		0	69.05	0		342.03	
MAN4		163,321	384,601		139,877	323,551	0	85.65	84.13		321.11	317
MAN5	647,066	255,500	222,599	530,862	204,610	183,022	82.04	80.08	82.22	320	326	329.37
MAN6	379,557	421,128	164,532	249,293	328,535	113,976	65.68	78.01	69.27	310	355.82	314.53
MAN7	91,875	317,294	478,463	80,536	286,223	421,307	87.66	90.21	88.05	334.81	334.42	334.34
MAN8	18,224	36,110	46,077	10,826	23,490	34,372	59.41	65.05	74.6	330.15	329.95	333.78
MAN9	46,321	74,321	49,106	36,404	59,342	39,904	78.59	79.85	81.26	331.09	331.42	332.08
MAN10	3,362	81	1,647	2,431	62	1,358	72.31	76.54	82.45	331.49	323.82	323.17
MAN11	74,089	76,591	102,795	46,618	52,182	70,736	62.92	68.13	68.81	322.13	324.99	328.5
MAN12	3	70		2	19		66.67	27.14	0	331.5	339	
MAN13	91,300	42,411	69,505	74,523	34,313	56,016	81.62	80.91	80.59	319.6	318.88	320.82
MAN14		5			5		0	100	0		329.8	
MAN15		40			27		0	67.5	0		321.96	
MAN16	4		2	4		1	100	0	50	325.5		217
MAN17	322,983	202,600	146,422	241,505	161,182	115,869	74.77	79.56	79.13	324.33	327.12	325.3
MAN18			117			81	0	0	69.23			323.2
MAN19	88,480	111,803	79,831	59,039	76,456	49,011	66.73	68.38	61.39	329.38	326.78	324.52
MAN20	1	8	2	0	5	1	0	62.5	50		336.8	372
MAN21	3	6,959		1	5,854		33.33	84.12	0	353	326.69	

Sample no	Input sequences			Good sequences (frequency)			Good sequences (%)			Good sequences mean length		
	1	2	3	1	2	3	1	2	3	1	2	3
MAN22	285,808	163,412	176,316	224,913	114,144	139,854	78.69	69.85	79.32	329.33	316.12	333.26
MAN23	189,679	243,920	300,872	154,801	196,927	241,129	81.61	80.73	80.14	321	326	329.92
MAN24	1,211,928	1,127,893	411,432	947,770	906, 247	304,371	78.2	80.34	73.98	355.15	352.00	349
MAN25	112,557	9,107	5,587	93,554	7,427	4,487	83.12	81.55	80.31	329.72	329.63	329.92
MAN26	533,646	234,226	63,669	356,778	168,175	47,896	66.86	71.8	75.23	345	337.99	355.07
MAN27	277,725	234,226	319,745	171,501	168,175	226,278	61.75	71.8	70.77	277	337.99	338.18
MAN28	148,593	437,038	270,599	102,927	296,224	188,778	69.27	67.78	69.76	319.21	319.81	316.82
MAN29	39,637	38,328	15,551	11,697	11,978	5,365	29.51	31.25	34.5	300.85	308.2	308.75
MAN30	44,036	49,533	62,454	33,283	39,318	49,984	75.58	79.38	80.03	325.74	328.76	328.32
MAN31	247,288	146,327	202,495	211,274	125,347	172,744	85.44	85.66	85.31	315	313.08	315.76
MAN32		1,780	8,504		1,230	5,581	0	69.1	65.63		335.19	337.38
MAN33	116,806	2,253	28,622	88,685	1,743	21,049	75.93	77.36	73.54	328.13	330.16	330.21
MAN34	83,758	42,058	37,741	63,196	32,078	29,872	75.45	76.27	79.15	320.03	319.67	325.89
MAN35	154,010	253,242	238,428	133,348	225,254	206,110	86.58	88.95	86.45	313.59	312.53	312.86
MAN36	6	88		5	48		83.33	54.55	0	323.4	336.88	
MAN37	25,040	16,606	22,210	18,535	11,599	16,004	74.02	69.85	72.06	334.73	332.99	334.32
MAN38	2		2	1		0	50	0	0	344		
MAN39	111,775	92,079	34,741	92,228	73,954	27,282	82.51	80.32	78.53	322.85	323.95	323.96
MAN40	2	4	6	1	2	5	50	50	83.33	323	339	344.4
MAN41	227,063	210,022	190,999	171,724	160,837	143,650	75.63	76.58	75.21	332.42	331.8	332.2
MAN42	720,225	253,549	732,408	582,223	193,914	578,310	80.84	76.48	78.96	331.68	332.43	333.31
MAN43	99,542	93,461	200,945	83,426	75,773	168,675	83.81	81.07	83.94	331.88	326.72	330.89
MAN44	1			1			100	0	0	312		
MAN45	7,738	9,901	14,937	5,947	7,216	11,181	76.85	72.88	74.85	301.13	301.08	302.1

Sample no	Input sequences			Good sequences (frequency)			Good sequences (%)			Good sequences mean length		
	1	2	3	1	2	3	1	2	3	1	2	3
MAN46	114,966	132,534	190,421	64,241	74,600	87,732	55.88	56.29	46.07	342.1	338.38	338.71
MAN47	42,739	163,329	121,176	32,260	121,673	87,792	75.48	74.5	72.45	323.92	324.32	322.26
MAN48	28,310	205,607	95,549	17,651	131,073	61021	62.35	63.75	63.86	308.43	310.97	308.73
MAN49	168,664	144,128	453,167	115,415	100,384	304,076	68.43	69.65	67.1	327.89	327.47	328.36
MAN50	12,158	19,274	15,068	7,473	10,841	10,161	61.47	56.25	67.43	308.58	319.21	308.64
MAN51	10,662	21,282	16,984	8,024	16,759	12,381	75.26	78.75	72.9	327.39	328.36	328.36
MAN52	307,688	246,450	246,048	234,390	190,165	193,344	76.18	77.16	78.58	320.51	321.82	321.42
MAN53							0	0	0			
MAN54	323,734	155,303	147,256	246,829	112,895	109,800	76.24	72.69	74.56	321	317.78	323.96
MAN55	113,694	70,036	50,476	99,749	62,703	44,635	87.73	89.53	88.43	333.9	334.03	334.18
MAN56	10,721	11,010	13,104	7,557	7,921	9,258	70.49	71.94	70.65	339.44	339.04	342.95
MAN57	1,518	9,986	20,844	710	4,392	8,290	46.77	43.98	39.77	329.52	330.33	331.86
MAN58	69,170	60,692	52,047	50,968	43,724	40,228	73.69	72.04	77.29	321.77	321.62	321.2
MAN59	2,117	34,331	35,293	1,456	20,110	21,697	68.78	58.58	61.48	326.92	323.19	320.2
MAN60	55,527	292,443	434,663	49,759	268,038	389,839	89.61	91.65	89.69	322.13	322.08	322.31
MAN61	64,212	322,025	477,105	57,177	285,601	411,060	89.04	88.69	86.16	323.25	322.43	323.19
MAN62	1	3		0	1		0	33.33	0		295	

Key: 1, 2 and 3 represents replica 1, 2 and 3 respectively. Blanks represent replicas that did not produce quality sequences.

The Ion-Torrent runs of the analysed 47 mixture samples generated between 45 and 938,751 reads after filtering. The clustering yielded 1 – 211 MOTUs per replicate per sample (Table 4.12). There is a significant positive relation between the number of reads generated and number of motus produced, r (45) = 0.41, $p < 0.001$ calculated based on means, (Appendix VI).

Out of the 47 analysed mixture samples, 74 % (35) had undeclared fillers, while the rest 26 % had no fillers. The fillers considered in this study were:*Zea mays* L. (Maize), *Sorghum bicolor* (L.) Moench (Sorghum), *Oryza sativa* L. (Rice) and *Pennisetum glaucum* (L.) R. Br. (millet) and *Triticum Spp*. Triticum spp was considered as filler when it was detected but not listed as species in the mixture.

Table 4.12: Summary data for MOTUs produced after sequence filtering (pages 89 to 91)

S no	Product Name	nrITS2 - Sequence yield in triplicates after filtering			nrITS2 - No. of MOTU's in triplicates before NCBI BLAST identification			nrITS2 - No. of MOTU's in triplicates after NCBI BLAST identification		
		No. of reads in replicate 1 (MOTU's)	No. of reads in replicate 2 (MOTU's)	No. of reads in replicate 3 (MOTU's)	No. of MOTU's in replicate 1 (MOTU's)	No. of MOTU's in replicate 2 (MOTU's)	No. of MOTU's in replicate 3 (MOTU's)	1	2	3
1	MAN2	51782	62348	45929	71	92	73	46	68	48
2	MAN4	0	138821	321837	0	42	87	0	30	69
3	MAN5	520304	198879	178561	153	91	79	133	81	73
4	MAN6	243408	110732	110513	185	122	116	148	97	92
5	MAN7	79551	283719	11591	17	35	57	17	33	42
6	MAN8	10109	22490	32574	22	39	49	15	29	32
7	MAN9	35491	58055	38837	11	30	20	10	23	18
8	MAN10	2379	45	1235	15	1	5	14	1	5
9	MAN11	44951	50495	68067	70	55	74	64	53	67
10	MAN13	73889	34042	55580	17	6	14	13	3	11
11	MAN17	237394	157684	112860	95	73	67	87	70	60
12	MAN19	55599	72484	44634	70	97	57	52	71	35
13	MAN22	218940	120640	135679	122	85	90	105	76	78
14	MAN23	153466	195004	239363	83	116	110	68	89	92
15	MAN24	938751	897822	301766	50	54	23	36	37	18
16	MAN25	90493	6876	4141	74	18	15	61	15	12
17	MAN26	354574	325725	47422	19	16	4	18	16	4
18	MAN27	168266	164761	222242	77	48	66	35	19	25

S no	Product Name	nrITS2 - Sequence yield in triplicates after filtering			nrITS2 - No. of MOTU's in triplicates before NCBI BLAST identification			nrITS2 - No. of MOTU's in triplicates after NCBI BLAST identification		
		No. of reads in replicate 1 (MOTU's)	No. of reads in replicate 2 (MOTU's)	No. of reads in replicate 3 (MOTU's)	No. of MOTU's in replicate 1 (MOTU's)	No. of MOTU's in replicate 2 (MOTU's)	No. of MOTU's in replicate 3 (MOTU's)	1	2	3
19	MAN28	100764	291563	185353	72	169	137	53	127	103
20	MAN29	10931	11140	5022	28	29	16	25	25	14
21	MAN30	32005	38102	418243	28	39	45	27	36	34
22	MAN31	206958	122638	168872	66	48	71	47	33	56
23	MAN32	0	1160	5373	0	7	17	0	7	17
24	MAN33	86418	1135	20066	107	6	35	72	4	28
25	MAN34	62014	185353	29107	81	137	58	50	39	39
26	MAN35	131096	219902	201941	70	92	85	64	82	79
27	MAN37	17818	11375	6879	44	38	41	34	31	32
28	MAN39	90221	72211	26564	67	60	31	51	48	23
29	MAN41	169538	158308	141555	67	83	46	56	68	37
30	MAN42	574120	189870	571570	211	74	148	159	59	114
31	MAN43	82198	74597	166447	73	68	107	57	52	81
32	MAN45	5116	6183	9916	16	23	27	10	14	18
33	MAN46	64017	74258	86785	21	25	27	19	23	26
34	MAN47	27924	111169	78304	58	109	76	40	70	46
35	MAN48	16807	129682	60119	6	53	17	5	50	17
36	MAN49	112479	96252	296964	97	66	126	91	60	120
37	MAN50	7038	9447	9824	36	32	36	36	30	36
38	MAN51	7450	15507	11591	37	65	54	30	46	44
39	MAN52	232755	189216	192333	41	51	47	34	42	38

S no	Product Name	nrITS2 - Sequence yield in triplicates after filtering			nrITS2 - No. of MOTU's in triplicates before NCBI BLAST identification			nrITS2 - No. of MOTU's in triplicates after NCBI BLAST identification		
		No. of reads in replicate 1 (MOTU's)	No. of reads in replicate 2 (MOTU's)	No. of reads in replicate 3 (MOTU's)	No. of MOTU's in replicate 1 (MOTU's)	No. of MOTU's in replicate 2 (MOTU's)	No. of MOTU's in replicate 3 (MOTU's)	1	2	3
40	MAN54	239592	108862	105936	136	81	77	110	73	61
41	MAN55	97653	61024	43481	34	19	18	33	19	17
42	MAN56	7141	7656	8985	24	21	19	22	19	19
43	MAN57	389	3642	6931	3	39	70	3	34	59
44	MAN58	48849	42431	39318	43	61	48	39	56	44
45	MAN59	993	19241	20517	6	51	58	3	38	35
46	MAN60	48749	264714	384838	9	20	52	7	17	43
47	MAN61	54642	280948	405397	28	63	134	21	50	99

Analysis at family level indicate that out of the 47 analysed mixed species samples, only 17 samples (36 %) had all the families of the species listed on the label while 2 samples (4 %) (MAN47 and MAN55) had none of the families of the listed species as reflected in Table 4.13. One sample (MAN32) did not include any added family, whereas the others had 1 - 11 families not declared on the labels but were detected using DNA metabarcoding analysis.

Table 4.13: Authenticity analysis at family level for mixed samples (pages 93 to 95)

Sample No.	Sample code	Total no. of family mentioned in labels	Total no. detected by DNA meta barcoding	Total no. identified at family (numbers)	Total no. identified at family level (% absolute)	Total no. not identified at family level (numbers)	Not identified at family (% relative)	Total no. identified but not listed
1	MAN5	2	3	2	100	0	0	1
2	MAN8	3	7	3	100	0	0	4
3	MAN11	12	18	12	100	0	0	6
4	MAN17	10	15	10	100	0	0	5
5	MAN25	5	10	5	100	0	0	5
6	MAN30	8	13	8	100	0	0	5
7	MAN31	7	15	7	100	0	0	8
8	MAN33	5	12	5	100	0	0	7
9	MAN34	5	12	4	80	1	20	7
10	MAN35	6	13	6	100	0	0	7
11	MAN39	2	3	2	100	0	0	1
12	MAN42	10	13	10	100	0	0	3
13	MAN43	7	10	7	100	0	0	3
14	MAN48	3	4	3	100	0	0	1
15	MAN56	6	9	6	100	0	0	3
16	MAN58	4	11	4	100	0	0	7
17	MAN59	4	11	4	100	0	0	7
18	MAN60	4	5	4	100	0	0	1
19	MAN28	8	15	7	88	1	13	8
20	MAN61	8	13	7	88	1	13	6

Sample No.	Sample code	Total no. of family mentioned in labels	Total no. detected by DNA meta barcoding	Total no. identified at family (numbers)	Total no. identified at family level (% absolute)	Total no. not identified at family level (numbers)	Not identified at family (% relative)	Total no. identified but not listed
21	MAN41	7	9	6	86	1	14	3
22	MAN49	7	13	6	86	1	14	7
23	MAN6	6	12	5	83	1	17	7
24	MAN19	5	10	4	80	1	20	6
25	MAN50	5	11	4	80	1	20	7
26	MAN7	4	6	3	75	1	25	3
27	MAN9	4	4	3	75	1	25	1
28	MAN27	4	5	3	75	1	25	2
29	MAN32	4	3	3	75	1	25	0
30	MAN45	4	8	3	75	1	25	5
31	MAN52	4	6	3	75	1	25	3
32	MAN57	4	6	3	75	1	25	3
33	MAN37	7	10	5	71	1	14	5
34	MAN23	3	10	2	67	1	33	8
35	MAN26	3	4	2	67	1	33	2
36	MAN29	3	3	2	67	1	33	1
37	MAN2	2	12	1	50	1	50	11
38	MAN4	2	8	1	50	1	50	7
39	MAN10	2	2	1	50	1	50	1
40	MAN22	6	14	3	50	3	50	11
41	MAN54	8	6	4	50	4	50	2
42	MAN13	3	4	1	33	2	67	3
43	MAN24	3	6	1	33	2	67	5
44	MAN46	3	2	1	33	2	67	1

Sample No.	Sample code	Total no. of family mentioned in labels	Total no. detected by DNA meta barcoding	Total no. identified at family (numbers)	Total no. identified at family level (% absolute)	Total no. not identified at family level (numbers)	Not identified at family (% relative)	Total no. identified but not listed
45	MAN51	6	7	2	33	4	67	5
46	MAN47	9	9	0	0	9	100	9
47	MAN55	3	1	0	0	3	100	1

Authenticity analyses at species level indicate that 16 out of the analyzed 47 samples (34 %) had all the species present in the product as listed on the label even though they also included other undeclared species (contaminated). Two samples (MAN 47 and MAN 55) (4 %) did not contain any of the species as listed on the label thus substituted. The rest of the samples (29) did not contain some of the listed species as shown in Table 4.14. There was positive correlation between total detected species and adulteration species recorded, $r(45) = 0.86$, $p<0.001$ (Appendix VII).

Table 4.14: Authenticity summary results of the analyzed 47 samples at species level using DNA metabarcoding (pages 97 to 99)

S. No	Product code	No. of Species on label	No. Detected	Fidelity (Expected - detected, absolute)	Fidelity - relative	Adulteration (Detected-Not expected- absolute)	Adulteration - relative
1	MAN 5	2	4	2	100.00	2	50.00
2	MAN 8	3	7	3	100.00	4	57.14
3	MAN 11	13	23	13	100.00	10	43.48
4	MAN 17	10	18	10	100.00	8	44.44
5	MAN 30	8	14	8	100.00	6	42.86
6	MAN 31	7	17	7	100.00	10	58.82
7	MAN 33	5	14	5	100.00	9	64.29
8	MAN 35	7	17	7	100.00	10	58.82
9	MAN 39	3	7	3	100.00	4	57.14
10	MAN 42	12	16	12	100.00	4	25.00
11	MAN 43	8	13	8	100.00	5	38.46
12	MAN 48	3	4	3	100.00	1	25.00
13	MAN 56	6	12	6	100.00	6	50.00
14	MAN 58	4	14	4	100.00	10	71.43
15	MAN 59	4	13	4	100.00	9	69.23
16	MAN 60	4	6	4	100.00	2	33.33
17	MAN 28	8	21	7	87.50	14	66.67
18	MAN 49	8	16	7	87.50	9	56.25
19	MAN 61	8	14	7	87.50	7	50.00
20	MAN 25	7	12	6	85.71	6	50.00
21	MAN 34	7	17	6	85.71	11	64.71

S. No	Product code	No. of Species on label	No. Detected	Fidelity (Expected - detected, absolute)	Fidelity - relative	Adulteration (Detected-Not expected- absolute)	Adulteration - relative
22	MAN 37	7	12	6	85.71	6	50.00
23	MAN 41	7	10	6	85.71	4	40.00
24	MAN 6	6	19	5	83.33	14	73.68
25	MAN 19	6	14	5	83.33	9	64.29
26	MAN 50	6	15	5	83.33	10	66.67
27	MAN 57	5	7	4	80.00	3	42.86
28	MAN 7	4	6	3	75.00	3	50.00
29	MAN 9	4	4	3	75.00	1	25.00
30	MAN 27	4	7	3	75.00	4	57.14
31	MAN 32	4	3	3	75.00	0	0.00
32	MAN 45	4	8	3	75.00	5	62.50
33	MAN 52	4	6	3	75.00	3	50.00
34	MAN 10	3	3	2	66.67	1	33.33
35	MAN 23	3	11	2	66.67	9	81.82
36	MAN 26	3	4	2	66.67	2	50.00
37	MAN 29	3	3	2	66.67	1	33.33
38	MAN 2	2	14	1	50.00	13	92.86
39	MAN 4	2	8	1	50.00	7	87.50
40	MAN 54	8	8	4	50.00	4	50.00
41	MAN 22	7	16	3	42.86	13	81.25
42	MAN 13	3	4	1	33.33	3	75.00
43	MAN 24	3	7	1	33.33	6	85.71

S. No	Product code	No. of Species on label	No. Detected	Fidelity (Expected - detected, absolute)	Fidelity - relative	Adulteration (Detected-Not expected- absolute)	Adulteration - relative
44	MAN 51	6	8	2	33.33	6	75.00
45	MAN 46	4	2	1	25.00	1	50.00
46	MAN 47	9	12	0	0.00	12	100.00
47	MAN 55	3	1	0	0.00	1	100.00

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Common species in trade across the three counties

In this study a total of 86 plant species were recorded to be traded as single species herbal product and 16 dominant families from the single species herbal products that were traded across the three counties. In each family there were a minimum of two species with the family Fabaceae having the highest number of 17 species and it constituted the most herbal products analysed. Fourty four out of the 86 plant species were common across the three regions and belonged to 31 plant families.

The highest number of herbal plant species were recorded in Kajiado County, probably due to close proximity to conserved wild forests such as Ngong Hills , Oloolua, Tsavo reserves and a number of community conservancies such as Shompole community trust and Eselenkei where the herbal plants are collected.. These conserved areas could have contributed to the festablished that there was a low species diversity. However in the past Mau complex in Narok County was the main source of herbal plants (Ndegwa, 2008; Nankaya *et al*., 2020). The low species diversity reported in this study may be attributed to human invasion and establishment of agricultural activities which have contributed to destruction of herbal shrubs and trees.. At Nairobi county, there were a few recorded authentic herbal plant species probably because the herbalists rely on a few suppliers who may be undertaking adulteration of the herbal products with more fillers for self gain at the expense of consumers.

5.1.2 Sources of herbal products in the study area

Diverse sources of herbal products were recorded: shrubs at 66 % (57species), trees 14 (16 %) herbs 10 (12 %) and climbers 5 (6 %). The dominance of shrubs and trees in this study is in agreement with studies conducted in Mana Angetu district and Hawassa city in Southern Ethiopia (Lulekal *et al.*, 2008; Tefera and Kim, 2019; Assefa *et al.*, 2021), and in Kitui, Kenya (Mutie *et al.*, 2020) while a study in Pakistan had reported trees as the source of most traded herbal products followed by herbs (Rehman *et al.*, 2017). This study generated a list of plant species being traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties, thus hypothesis one was accepted.

5.1.3 Plant parts used and ailment treatment associations

This study revealed that stem, bark, leaves, roots, seeds and tubers were used in combination or as single species to treat ailments. The most utilized parts of plants were stems and barks. Most studies in the world have recorded leaves as the most traded plant part in herbal products preparations (Islam *et al*., 2014; Mukungu *et al*., 2016; Demie *et al*., 2018; Faruque *et al*., 2018; Venkatachalapathi *et al*., 2018; Krupa *et al*., 2019; Ndhlovu *et al*., 2019). Other studies carried out in Ethiopia such as Lulekal *et al*. (2008) and Kenya (Kiringe, 2006) showed that roots were the most utilized plant parts in preparation of herbal remedies followed by leaves. Single species were used to treat several ailments while single ailments could be treated by a variety of herbal species.

Osyris lanceolata had the highest percentage use index (68.9 %) and mostly used to treat abdominal and sexual ailments. *Rhamnus staddo*, *Rapanea melanophloes* and *Acacia nilotica* had percentage use index above 50% and used for treatment of the three

leading ailment groups (abdominal, respiratory and malaria). This is an indication that the probability of trade and use of these four species is above half and therefore the demand for them in the market is high.

5.1.4 Morphological and DNA taxonomic classification

Out of the 86 plant species recorded from single species samples, 44 were common across the counties. These common species were identified and confirmed using both taxonomic and DNA barcoding technology. However, identification of these samples using DNA barcoding technology was more accurate than the taxonomic method. Similar studies by Ghorbani *et al.* (2017) and Jamdade *et al.* (2022) reported that use of DNA barcoding technology was efficient is identification of herbal plant species and closely related species compared to morphological taxonomy.

The use DNA barcoding technology made it possible to identify forty four common species and group them into 31 families with Fabaceae, Apocynaceae and Meliaceae as the most identified species. Among the three families, Fabaceae had the highest number of herbal plant species while Meliaceae had the least. The Fabaceae, Apocynaceae and Rhamnaceae families were the most traded families as both single and mixed species herbal products. Fabaceae has also been reported to be the most traded plant family in herbal products in Madhupur forest area of Bangladesh (Islam *et al*., 2014); in India (Silambarasan *et al*., 2017 and Krupa *et al*., 2019); Tigray region of Ethiopia (Kidane *et al*., 2018); and in Tanzania (Hilonga *et al*., 2019).

Utilization of Rutaceae and Rhamnaceae as ethnomedicinal plants has been reported in parts of Italy, Pakistan and other parts of the world (Shinwari 2010; Bussa and

Belayneh, 2019; Mailu *et al*. 2020) similar to findings of this study. This can be attributed to its wide distribution in many environments. In addition, the study revealed that each County under study had specialized species listed and used as herbal species. This may be due to their specific habitat requirements.

Three species (Aloe sp, Osyris lanceolata and Prunus africana) were recorded in the study that are protected nationally and internationally. These species are traded illegally. All Aloe species are nationally protected in Kenya according to the 1986, presidential decree that declared a ban on harvesting of wild-growing aloes and gazettement of Aloe Utilization guidelines by the Kenya government in 2007 (Anonymous, 2007) Since 1995, *Prunus africana* species is protected in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) CITES Appendix II listing IUCN (2005) while and *Osyris lanceolata* is protected in Kenya by Legal Notice No 3176 of 2007 under the Forests Act, 2005 as well as being listed on Appendix II of CITES list of endangered species, for regulation of trade from wild harvest. However the study observed that none of the herbalists participating in this study had the necessary permits for either harvesting or trading the three protected plant species. The plant species commonly traded as herbal products in selected markets in Kajiado, Nairobi and Narok counties were identified using both botanical taxonomy and DNA barcoding techniques therefore hypothesis two was accepted.

5.1.5 Generation of reference barcode sequence using DNA technology

Reference barcode sequences were generated for 38 of the common traded herbal plant species together with their closely related species and deposited to the GenBank. The DNA Technology was able to produce quality sequences from the herbal plants voucher

specimen collected. The barcode reference database created using taxonomically resolved and curated voucher specimens was successful in identifying and verifying herbal plant species in the herbal products analyzed. The reference database supplemented the sequence data available in Genbank that is usually frequently used as reference data since most of the listed herbal species from Kenya were under-represented. Based on these findings, the third hypothesis was accepted.

5.1.6 Authenticity of herbal products traded in selected markets using DNA barcoding technology

This study has shown successful rates of extracting DNA from dried, ground and powdered herbal products on sale in selected Kenyan markets for molecular studies. The DNA extracted from fresh collected voucher specimens for reference library had better quality compared to that extracted from market herbal samples. These results are similar to those reported by Ragupathy *et al*. (2019) and Abubakar *et al*. (2021). The difference between the quality of fresh specimens and market samples could be due to degradation and fragmentation of DNA during drying, grinding and packaging of the herbal products.

Among the markers *ITS* had the highest identification efficiency compared to *rbcL* and *MatK*. Similar results have been reported by Sheidai *et al*. (2019) where *ITS* was used to distinguish *Ziziphora* species in Iran, and in China in molecular authentication of the herbal products of Liguisticum sp. usually sold as dried sliced material (Liu *et al*., 2019).

When *ITS* and *rbcL* were combined, it was possible to identify most of the samples to species level while fewer samples were confirmed using the combination of *ITS* and *matk.* Previous studies have established that the combination of different DNA barcode markers is necessary for adequate species verification (Tnah *et al.*, 2019; Zhang and Jiang, 2020). In Canada, the species from native temperate flora of Ontario were tested using *rbcL*, matK and trnHPsbA barcodes that had 95.3 % identification success (Burgess *et al*., 2011).

Kajiado County had more authentic samples in comparison to Narok and Nairobi probably because they have access to forest as compared to Narok where most of the natural habitats like Mau complex have been encroached and cleared for agricultural activities. On the other hand, Nairobi being the capital city is highly populated and there is likelihood of adulteration for economic gain.

Single species samples were more authentic compared to the mixed samples. In single species samples, adulterations of most of the species listed was mainly by substitution with closely related or look-alike non-herbal and herbal plant species. Substitution with the wrong herbal species leads to many herbal products sold not being authentic. These results are similar to those recorded in Malaysia by (Tnah *et al*., 2019) where herbal products on labels were found to be substituted with herbal plant species carrying similar vernacular names. This can be attributed to confusing closely related species with similar vernacular names that are normally hard to distinguish during wild collection.

5.1.7 Authentication of mixed species samples using DNA metabarcoding

DNA metabarcoding technology was successful in mixed species samples identification at 92% to species level and 4 % to Genus and family levels respectively.

This concurs with other previous studies where the technology has been used to identify and confirm herbal product plant species (Staats *et al*., 2016; Raclariu *et al*., 2017; Xin *et al*., 2018; Lo and Shaw, 2019; Seethapathy *et al*., 2019; Zhang *et al*., 2019; Urumarudappa *et al*., 2020). Further, validation of mislabeling in fresh and processed commercialized herbal products have also applied DNA metabarcoding technology successfully (Sheth and Thaker, 2015; Guo *et al*., 2017; Molina *et al*., 2018; Veldman *et al*., 2020).

In the present study, DNA metabarcoding has shown efficiency in identifying plant species in mixed species herbal samples where a total of 96 plant species were identified to species level an indication of high diversity from the studied counties. Several studies have shown the technology can be applied for species diversity identification in herbal and supplement products (Ivanova *et al*., 2016; Zhang and Jiang, 2020; Le *et al*., 2020).

Most of the 47 analyzed mixed species samples had adulteration and substitution of species as detected using DNA technology. The discrepancies between the listed species on labels and the detected species, raises concern on quality and authenticity of the herbal products sold in the study area. Some of the substitution was by species that have no medical correlation to those listed and similar results were recorded in Brazil (Palhares *et al*., 2021) where the Brazilian quina (*Remijia ferruginea* (A.St.-Hil.) DC. and *Strychnos pseudoquina* (A. St.-Hil.) barks were substituted by other species that had no medicinal value. Such adulteration and substitution in herbal products poses a risk to consumers (Gakuya *et al*., 2020). These results are similar to those reported by Sgamma *et al*. (2017), Howard *et al*. (2019) and Seethapathy *et al*. (2019).

Maize (*Zea mays*) was the most used undeclared filler in 74 % of the analyzed samples. Presence of undeclared fillers such as maize, rice and wheat pose a health concern to people with various allergies. Even though contamination and substitution is by other herbal species, lack of understanding of herbal products interaction may result to adverse effects on users. Adulteration and substitution could have as well been done knowingly to increase the revenue at the expense of the health of users.

Most of the traded plant species identified using barcoding technology and taxonomic methods belong to the Fabaceae family. These results are in line with studies carried out in Tanzania by Hilonga *et al*. (2019) where Fabaceae was recorded as most traded. As a result, hypothesis four does not hold since plants herbal products analyzed in the study area were not accurately labeled.

5.2 Conclusions

i. Selected markets in Kajiado, Nairobi and Narok counties had 44 common plant species that were traded as herbal products.
ii. DNA barcoding technology is more reliable in identification of plant species used as herbal products compared to taxonomic technique.
iii. The study was able to create reference barcode sequences for herbal plant species using DNA technology.
iv. The study established that some of the plants herbal products were not accurately labeled.

5.3 Recommendations

i. DNA barcoding should be applied in identification and verification of herbal products since it is more accurate compared to taxonomic techniques in

identifying close relatives of the plant species and plants with different vernacular names for quality control.

ii. Reference library for herbal plant species developed in this study using barcoding technology can be applied in pharmacological and drug development studies.

iii. Further studies are recommended to assess authenticity of herbal products using DNA barcoding in other regions.

iv. More studies are recommended on herbal plant species collection and supply chains to deduce at what point contaminations and substitutions take place.

5.4 Further studies

Research on the following areas is recommended for further studies:

i. There is need to use DNA markers to establish if herbal products sold in the other counties in Kenya are collectly labelled for the safety of the consumers.

ii. A study should be carried out along the supply chain to establish where adulteration and substitution takes place.

REFERENCES

Abubakar, M. B., Salleh, M. F., Omar, S. M. S. and Wagiran, A. (2017). DNA barcoding and chromatography fingerprints for the authentication of botanicals in herbal medicinal products. *Evidence-Based Complementary and Alternative Medicine,* 2017, 1–28. *https://doi.org/10.1155/2017/1352948*

Abubakar, B. M., Salleh, F. M., Shamsir Omar, M. S. and Wagiran, A. (2018). Assessing product adulteration of *Eurycoma longifolia* (Tongkat Ali) herbal medicinal product using DNA barcoding and HPLC analysis. *Pharmaceutical Biology, 56(1*), 368–377. *https://doi.org/10.1080/13880209.2018.1479869*

Abubakar, B. M., Salleh, F., Wagiran, A. and Abba, M. (2021). Comparative evaluation of different DNA extraction methods from *E. longifolia*. *Herbal Medicinal Product, 2,* 21–26.

Agnew, A. D. (2013). Upland Kenya wild flowers and ferns: A flora of the flowers, ferns, grasses, and sedges of highland Kenya. *Nature Kenya-The East Africa Natural History* Society. 3rd edition, 9789966761170

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215(3),* 403–410. *https://doi.org/10.1016/S0022-2836 (05)80360-2*

Anonymous (2007) Wildlife (Conservation and management) (*Aloe* species) Regulations. (Cap. 376) [L.N. 403/2007]. Available online (http://faolex.fao.org/docs/pdf/ken101511.pdf). Accessed 21 May, 2021.

Anthoons, B., Karamichali, I., Schrøder-Nielsen, A., Drouzas, A. D., de Boer, H. and Madesis, P. (2021). Metabarcoding reveals low fidelity and presence of toxic species in short chain-of-commercialization of herbal products. *Journal of Food Composition and Analysis, 97,* 103767.

Asahina, H., Shinozaki, J., Masuda, K., Morimitsu, Y. and Satake, M. (2010). Identification of medicinal *Dendrobium* species by phylogenetic analyses using matK and rbcL sequences. *Journal of Natural Medicines, 64(2),* 133–138. *https://doi.org/10.1007/s11418-009-0379-8*

Assefa, B., Megersa, M. and Jima, T. T. (2021). Ethnobotanical study of medicinal plants used to treat human diseases in Gura Damole District, Bale Zone, South East Ethiopia. *Asian Journal of Ethnobiology*, *4*(1).

Beentje, H., Adamson, J. and Bhanderi, D. (1994). Kenya trees, shrubs, and lianas. National Museums of Kenya, 9966-9861-0-3

Bent, S. (2008). Herbal medicine in the United States: review of efficacy, safety, and regulation. *Journal of general internal medicine*, *23*(6), 854-859. https://doi.org/10.1007/s11606-008-0632-y

Burgess, K. S., Fazekas, A. J., Kesanakurti, P. R., Graham, S. W., Husband, B. C., Newmaster, S. G., Percy, D. M., Hajibabaei, M. and Barrett, S. C. (2011). Discriminating plant species in a local temperate flora using the rbcL+ matK DNA barcode. *Methods in Ecology and Evolution*, *2(4)*, 333-340. https://doi.org/10.1111/j.2041-210X.2011.00092.x

Bussa, N. F. and Belayneh, A. (2019). Traditional medicinal plants used to treat cancer, tumors and inflammatory ailments in Harari region, Eastern Ethiopia. *South African Journal of Botany*, *122*, 360-368.

Bussmann, R. W., Gilbreath, G. G., Solio, J., Lutura, M., Lutuluo, R., Kunguru, K., Wood N. and Mathenge, S. G. (2006). Plant use of the Maasai of Sekenani valley, Maasai Mara, Kenya. *Journal of Ethnobiology and Ethnomedicine, 2(1),* 22. *https://doi.org/10.1186/1746-4269-2-22*

Campbell, D. J., Gichohi, H., Mwangi, A. and Chege, L. (2000). Land use conflict in Kajiado District, Kenya. Land Use Policy, 17(4), 337–348. https://doi.org/10.1016/S0264-8377 (00)00038-7

Canter, P. H. and Ernst, E. (2004a). Anthocyanosides of *Vaccinium myrtillus* (Bilberry) for night vision - A systematic review of placebo-controlled trials. *Survey of Ophthalmology, 49(1), 38–50. https://doi.org/10.1016/j.survophthal.2003*.10.006

Canter, P. H., and Ernst, E. (2004b). Herbal supplement use by persons aged over 50 years in Britain. *Drugs and Aging, 21(9),* 597–605. *https://doi.org/10.2165/00002512-200421090-00004*

Chen, S. L., Yu, H., Luo, H. M., Wu, Q., Li, C. F. and Steinmetz, A. (2016). Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chinese medicine*, *11*(1), 1-10. doi: 10.1186/s13020-016-0108-7

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L Zhu, Y., Ma, X., Gao, T., Pang, X, Luo, K, Li, Y., Li, X.,Jia, X., Lin, Y. and Leon, C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE, 5(1), e8613. https://doi.org/10.1371/journal.pone.0008613*

Cheng, X., Su, X., Chen, X., Zhao, H., Bo, C., Xu, J., Bai, H. and Ning, K. (2014). Biological ingredient analysis of traditional Chinese medicine preparation based on high-throughput sequencing: the story for Liuwei Dihuang Wan. *Scientific reports, 4(1),* 1-12.

Coghlan, M. L., Haile, J., Houston, J., Murray, D. C., White, N. E., Moolhuijzen, P., Bellgard, M.I. and Bunce, M. (2012). Deep sequencing of plant and animal DNA contained within traditional Chinese medicines reveals legality issues and health safety concerns. *PLoS genetics, 8(4),* e1002657.

Cohen, P. A. and Ernst, E. (2010). Safety of herbal supplements: A guide for cardiologists. *Cardiovascular Therapeutics. 28(4), 246–253. https://doi.org/10.1111/j.1755-5922.2010.00193.x*

Cristescu, M. E. (2014). From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. *Trends in Ecology and Evolution, 29(10),* 566–571. *https://doi.org/10.1016/j.tree.2014.08.001*

Crossley, B. M., Bai, J., Glaser, A., Maes, R., Porter, E., Killian, M. L., Clement, T. and Toohey-Kurth, K. (2020). Guidelines for Sanger sequencing and molecular assay monitoring. Journal of Veterinary *Diagnostic Investigation, 32(6),* 767-775.

Da-cheng, H., Shi-lin, C., Pei-gen, X. and Yong, P. (2010). Authentication of medicinal plants by DNA-based markers and genomics. *Chinese Herbal Medicine, 2(4),* 250–261. *https://doi.org/10.3969/j.issn.1674-6384.2010.04.003*

Dalton, D. L. and Kotze, A. (2011). DNA barcoding as a tool for species identification in three forensic wildlife cases in South Africa. *Forensic Science International, 207(1–**3), e51–e54. https://doi.org/10.1016/j.forsciint.2010.12.017***

De Boer, H. J., Ghorbani, A., Manzanilla, V., Raclariu, A. C., Kreziou, A., Ounjai, S., Osathanunkul, M. and Gravendeel, B. (2017). DNA metabarcoding of orchid-derived products reveals widespread illegal orchid trade. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1863), 20171182.

de Vere, N., Rich, T. C. G., Ford, C. R., Trinder, S. A., Long, C., Moore, C. W., Satterthwaite, D., Davies, H., Allainguillaume, J., Ronca, S. and Tatarinova, T., Garbett, H., Walker. K. and Wilkinson, M. J. (2012). DNA barcoding the native flowering plants and conifers of Wales. *PLoS ONE, 7(6), e37945. https://doi.org/10.1371/journal.pone.0037945*

Deck, J., Gross, J., Stones-Havas, S., Davies, N., Shapley. R. and Meyer, C. (2012). Field information management systems for DNA barcoding. In *DNA Barcodes* Humana Press, Totowa, NJ, pp 255-267

Demie, G., Negash, M. and Awas, T. (2018). Ethnobotanical study of medicinal plants used by indigenous people in and around Dirre Sheikh Hussein heritage site of South-eastern Ethiopia. *Journal of Ethnopharmacology*, *220,* 87-93.

Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods, 10(10), 996–998. https://doi.org/10.1038/nmeth.2604*

Edgar, R. C. (2016). UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv*, 081257.

Egan, A. N., Schlueter, J. and Spooner, D. M. (2012). Applications of next-generation sequencing in plant biology. *American Journal of Botany, 99(2),* 175-185.

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 4,* 177. *https://doi.org/10.3389/fphar.2013.00177*

Ernst, E. (2005). The efficacy of herbal medicine - An overview. *Fundamental and Clinical Pharmacology, 19(4), 405–409. https://doi.org/10.1111/j.1472-8206.2005.00335.x*

Ewing, B., Hillier, L., Wendl, M. C. and Green, P. (1998). Base-calling of automated sequencer traces using Phred. I. Accuracy Assessment. *Genome Research, 8(3),* 175–185. *https://doi.org/10.1101/gr.8.3.175*

Faruque, M. O., Uddin, S. B., Barlow, J. W., Hu, S., Dong, S., Cai, Q., Li, X. and Hu, X. (2018). Quantitative ethnobotany of medicinal plants used by indigenous communities in the Bandarban District of Bangladesh. *Frontiers in Pharmacology, 9,* 40. *https://doi.org/10.3389/fphar.2018.00040*

Flynn, J. M., Brown, E. A., Chain, F. J., MacIsaac, H. J. and Cristescu, M. E. (2015). Toward accurate molecular identification of species in complex environmental samples: testing the performance of sequence filtering and clustering methods. *Ecology and evolution, 5(11),* 2252-2266.

Gakuya, D. W., Okumu, M. O., Kiama, S. G., Mbaria, J. M., Gathumbi, P. K., Mathiu, P. M. and Nguta, J. M. (2020). Traditional Medicine in Kenya: Past and current status, challenges and the way forward. *Scientific African, e00360. https://doi.org/10.1016/j.sciaf.2020.e00360*

Gathier, G., van der Niet, T., Peelen, T., van Vugt, R. R., Eurlings, M. C. M. and Gravendeel, B. (2013). Forensic Identification of CITES protected slimming Cactus (Hoodia) using DNA barcoding. *Journal of Forensic Sciences, 58(6), 1467–1471. https://doi.org/10.1111/1556-4029.12184*

Ghorbani, A., Saeedi, Y. and De Boer, H. J. (2017). Unidentifiable by morphology: DNA barcoding of plant material in local markets in Iran. *PloS one, 12(4*), e0175722.

Gunjan, M., Naing, T. W., Saini, R. S., Ahmad, A., Naidu, J. R. and Kumar, I. (2015). Marketing trends and future prospects of herbal medicine in the treatment of various disease. *World Journal of Pharmaceutical Research, 4(9),* 132-155.

Guo, M., Ren, L. and Pang, X. (2017). Inspecting the true identity of herbal materials from Cynanchum using ITS2 barcode. *Frontiers in Plant Science, 8,* 1945. *https://doi.org/10.3389/fpls.2017.01945*

Guo, X., Wang, X., Su, W., Zhang, G. and Zhou, R. (2011). DNA barcodes for discriminating the medicinal plant *Scutellaria baicalensis* (Lamiaceae) and its adulterants. *Biological and Pharmaceutical Bulletin*, 34(8), 1198–1203. http://www.ncbi.nlm.nih.gov/pubmed/21804206

Han, J., Pang, X., Liao, B., Yao, H., Song, J. and Chen, S. (2016). An authenticity survey of herbal medicines from markets in China using DNA barcoding. *Scientific Reports, 6(1), 18723. https://doi.org/10.1038/srep18723*

Hasen, G. and Hashim, R. (2021). Current awareness of health professionals on the safety of herbal medicine and associated factors in the south west of Ethiopia. *Journal of Multidisciplinary Healthcare*, *14*, 2001.

Heubl G. (2013) DNA-Based Authentication of TCM-Plants: Current Progress and Future Perspectives. In: Wagner H., Ulrich-Merzenich G. (eds) Evidence and Rational Based Research on Chinese Drugs. Springer, Vienna. https://doi.org/10.1007/978-3-7091-0442-2_2

Hilonga, S., Otieno, J. N., Ghorbani, A., Pereus, D., Kocyan, A. and de Boer, H. (2019). Trade of wild-harvested medicinal plant species in local markets of Tanzania and its implications for conservation. *South African Journal of Botany*, *122*, 214-224.

Höft, M., Barik, S. K. and Lykke, A. M. (1999). Quantitative ethnobotany. Applications of multivariate and statistical analyses in ethnobotany. *People and Plants Working Paper*, *6*, 1-49.

Hollingsworth, P. M., Graham, S. W. and Little, D. P. (2011). Choosing and using a plant DNA barcode. *PloS One*, *6*(5), e19254. https://doi.org/10.1371/journal.pone.0019254

Howard, C., Hill, E., Kreuzer, M., Mali, P., Masiero, E., Slater, A. and Sgamma, T. (2019). DNA Authentication of St John's wort (*Hypericum perforatum* L.) commercial products targeting the ITS region. *Genes, 10(4),* 286. *https://doi.org/10.3390/genes10040286*

Howland, O. (2020). Fakes and chemicals: indigenous medicine in contemporary Kenya and implications for health equity. *International Journal for Equity in Health, 19(1),* 1-12.

Ichim, M. C., Häser, A. and Nick, P. (2020). Microscopic authentication of commercial herbal products in the globalized market: Potential and limitations. *Frontiers in Pharmacology, 11,* 876.

Islam, M. K., Saha, S., Mahmud, I., Mohamad, K., Awang, K., Uddin, S. J., Rahman, M.M. and Shilpi, J. A. (2014). An ethnobotanical study of medicinal plants used by tribal and native people of Madhupur forest area, Bangladesh. *Journal of Ethnopharmacology, 151(2),* 921-930.

IUCN (2005) 2005 IUCN Red list of threatened species. IUCN, Gland, Switzerland. Available online (http://www.redlist.org). Accessed 21 May 2021.

Ivanova, N. V., Kuzmina, M. L., Braukmann, T. W. A., Borisenko, A. V. and Zakharov, E. V. (2016). Authentication of herbal supplements using next-generation sequencing. *PLOS ONE, 11(5), e0156426. https://doi.org/10.1371/journal.pone.0156426*

Jamdade, R., Mosa, K. A., El-Keblawy, A., Al Shaer, K., Al Harthi, E., Al Sallani, M., ... Al Jasmi, M., Gairola, S., Shabana, H. and Mahmoud, T. (2022). DNA barcodes for accurate identification of selected medicinal plants (Caryophyllales): Toward barcoding flowering plants of the United Arab Emirates. *Diversity, 14(4),* 262.

Jeruto, P., Lukhoba, C., Ouma, G., Otieno, D., and Mutai, C. (2008). An ethnobotanical study of medicinal plants used by the Nandi people in Kenya. *Journal of Ethnopharmacology, 116(2),* 370-376.

Jinbo, U., Kato, T. and Ito, M. (2011). Current progress in DNA barcoding and future implications for entomology. *Entomological Science, 14(2),* 107–124. *https://doi.org/10.1111/j.1479-8298.2011.00449.x*

Jusu, A. and Sanchez, A. C. (2014). Medicinal plant trade in Sierra Leone: threats and opportunities for conservation. Economic Botany, 68(1), 16-29.

Kantor, M. (2009). The role of rigorous scientific evaluation in the use and practice of complementary and alternative medicine. *Journal of the American College of Radiology, 6(4), 254–262.* https://doi.org/10.1016/J.JACR.2008.09.012

Kariuki, P. M., Njoka, J. T., Saitabau, C. L. and Saitabau, H. S. (2016). Forest governance, livelihoods and resilience: the case of Loita forest (Entime e Naimina enkiyio), Narok County, Kenya. In: Bose P., van Dijk H. (eds) *Dryland Forests* Springer, Cham, pp 117-138 https://doi.org/10.1007/978-3-319-19405-9_6

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. and Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199*

Kenya National Bureau of Statistics (KNBS) (2019). Kenya Population and Housing Census Volume 1: Population by County and sub-County. Available online (https://www.knbs.or.ke/?wpdmpro=2019-kenya-population-and-housing-census-volume-i-population-by-County-and-sub-County). Accesses 9th January 2019

Khan, M. S. A. and Ahmad, I. (2019). Herbal medicine: current trends and future prospects. In: Khan, M. S. A., Ahmad, I., and Chattopadhyay, D. (eds) *New Look to Phytomedicine.* Academic Press, pp 3-13

Kidane, L., Gebremedhin, G. and Beyene, T. (2018). Ethnobotanical study of medicinal plants in Ganta Afeshum District, Eastern Zone of Tigray, Northern Ethiopia. *Journal of Ethnobiology and Ethnomedicine, 14(1),* 1-19.

Kimball, L. A. (1995). The biodiversity convention: how to make it work. *Vanderbilt. Journal of Transnational Law*, *28*, 763-776.

Kipkore, W., Wanjohi, B., Rono, H., and Kigen, G. (2014). A study of the medicinal plants used by the Marakwet Community in Kenya. *Journal of Ethnobiology and Ethnomedicine, 10, 24. https://doi.org/10.1186/1746-4269-10-24*

Kiringe, J. W. (2006). A survey of traditional health remedies used by the Maasai of Southern Kajiado District, Kenya. *Ethnobotany Research and Applications, 4,* 061-074.

Kisangau, D. P. and Herrmann, T. M. (2007). Utilization and conservation of medicinal plants used for primary health care in Makueni district, Kenya. *The International Journal of Biodiversity Science and Management*, *3*(3), 184-192. https://doi.org/10.1080/17451590709618172

Kokwaro, J. O. (2009). Medicinal plants of East Africa. Nairobi: University Press. https://doi.org/10.2307/2076505

Kress, W. J. (2017). Plant DNA barcodes: Applications today and in the future. *Journal of Systematics and Evolution, 55(4),* 291–307. *https://doi.org/10.1111/jse.12254*

Kress, W. J. and Erickson, D. L. (2007). A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. *PLoS One, 2*(6), e508.

Krupa, J., Sureshkumar, J., Silambarasan, R., Priyadarshini, K. and Ayyanar, M. (2019). Integration of traditional herbal medicines among the indigenous communities in Thiruvarur District of Tamil Nadu, India. *Journal of Ayurveda and Integrative Medicine, 10(1), 32. https://doi.org/10.1016/j.jaim.2017.07.013*

Lammers, Y., Peelen, T., Vos, R. A. and Gravendeel, B. (2014). The HTS barcode checker pipeline, a tool for automated detection of illegally traded species from high-throughput sequencing data. *BMC Bioinformatics*, *15*(1), 1-8. *https://doi.org/10.1186/1471-2105-15-44*

Lange, D. (2006). International trade in medicinal and aromatic plants: actors, volumes and commodities. *Frontis, 17,* 155-170.

Le, D. T., Zhang, Y. Q., Xu, Y., Guo, L. X., Ruan, Z. P., Burgess, K. S. and Ge, X. J. (2020). The utility of DNA barcodes to confirm the identification of palm collections in botanical gardens. *PloS One*, *15*(7), e0235569.

Li, F. W., Kuo, L. Y., Rothfels, C. J., Ebihara, A., Chiou, W. L., Windham, M. D. and Pryer, K. M. (2011). rbcL and matK earn two thumbs up as the core DNA barcode for ferns. *PLoS One*, *6*(10), e26597. *https://doi.org/10.1371/journal.pone.0026597*

Li, M., Cao, H., But, P. P. H. and Shaw, P. C. (2011). Identification of herbal medicinal materials using DNA barcodes. *Journal of Systematics and Evolution*, *49(3*), 271-283. *doi: 10.1111/j.1759-6831.2011.00132.x*

Li, W. and Godzik, A. (2006). Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics, 22(13),* 1658–1659. *https://doi.org/10.1093/bioinformatics/btl158*

Lindegårdh, N., Dondorp, A. M., Singhasivanon, P., White, N. J. and Day, N. P. J. (2007). Validation and application of a liquid chromatographic–mass spectrometric method for determination of artesunate in pharmaceutical samples. *Journal of Pharmaceutical and Biomedical Analysis, 45(1),* 149–153. *https://doi.org/10.1016/J.JPBA.2007.04.030*

Liu, Z. W., Gao, Y. Z. and Zhou, J. (2019). Molecular authentication of the medicinal species of *Ligusticum* (*Ligustici Rhizoma* et Radix,"Gao-ben") by integrating non-coding internal transcribed spacer 2 (ITS2) and its secondary structure. *Frontiers in Plant Science, 10*, 429. *https://doi.org/10.3389/fpls.2019.00429*

Lo, Y. T. and Shaw, P. C. (2019). Application of next-generation sequencing for the identification of herbal products. *Biotechnology Advances*, *37*(8), 107450. *https://doi.org/10.1016/j.biotechadv.2019.107450*

Loya, A. M., González-Stuart, A. and Rivera, J. O. (2009). Prevalence of polypharmacy, polyherbacy, nutritional supplement use and potential product interactions among older adults living on the United States-Mexico border. *Drugs and Aging*, *26(5),* 423-436.

Lulekal, E., Kelbessa, E., Bekele, T. and Yineger, H. (2008). An ethnobotanical study of medicinal plants in Mana Angetu District, Southeastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine, 4(1),* 1-10

Mailu, J. K., Nguta, J. M., Mbaria, J. M. and Okumu, M. O. (2020). Medicinal plants used in managing diseases of the respiratory system among the Luo community: an appraisal of Kisumu East Sub-County, Kenya. *Chinese Medicine, 15(1),* 1-27.

Maundu, P., Berger, D., Nasieku, J., Kipelian, M., Mathenge, S. G. and Morimoto, Y. (2001). Ethnobotany of the Loita Maasai. *People and Plants Working Paper*, *8*, 1–35. Available online

(http://www.rbgkew.org.uk/peopleplants/wp/wp8/index.html). Accessed 20th January 2018.

Mensah, M. L., Komlaga, G., Forkuo, A. D., Firempong, C., Anning, A. K. and Dickson, R. A. (2019). Toxicity and safety implications of herbal medicines used in Africa. *Herbal medicine, 63,* 1992-0849.

MoALF. (2018). Climate Risk Profile for Kajiado County. Kenya County Climate Risk Profile Series. The Kenya Ministry of Agriculture, Livestock and Fisheries (MoALF), Nairobi, Kenya. Available online (https://ccafs.cgiar.org/publications/climate-risk-profile-kajiado-County-kenya-County-climate-risk-profile-series#.XbgrDUYzaUk). Accesses 2nd August 2019.

Mohrbeck, I., Raupach, M. J., Martínez Arbizu, P., Knebelsberger, T. and Laakmann, S. (2015). High-throughput sequencing—the key to rapid biodiversity assessment of marine metazoa?. *PLoS One*, *10*(10), e0140342.

Molina, J., Sherpa, C., Ng, J., Sonam, T. and Stuhr, N. (2018). DNA barcoding of online herbal supplements: crowd-sourcing pharmacovigilance in high school. *Open Life Sciences, 13(1),* 48–55. *https://doi.org/10.1515/biol-2018-0007*

Mukungu, N., Abuga, K., Okalebo, F., Ingwela, R., and Mwangi, J. (2016). Medicinal plants used for management of malaria among the Luhya community of Kakamega East sub-County, Kenya. *Journal of Ethnopharmacology, 194,* 98-107.

Mutie, F. M., Rono, P. C., Kathambi, V., Hu, G. W. and Wang, Q. F. (2020). Conservation of wild food plants and their potential for combatting food insecurity in Kenya as exemplified by the drylands of Kitui County. *Plants, 9(8),* 1–25. *https://doi.org/10.3390/plants9081017*

Mwangi, J. and Gitonga, L. (2014). Perceptions and use of herbal remedies among patients with diabetes mellitus in Murang'a North District, Kenya. *Open Journal of Clinical Diagnostics, 4(03), 152–172. https://doi.org/10.4236/ojcd.2014.43024*

Mwaura, A., Kamau, J. and Ombori, O. (2020). An ethnobotanical study of medicinal plants commonly traded in Kajiado, Narok and Nairobi counties, Kenya: *East African Journal of Science, Technology and Innovation*, *1*(3). https://doi.org/10.37425/eajsti.v1i3.153

Nankaya, J., Nampushi, J., Petenya, S. and Balslev, H. (2020). Ethnomedicinal plants of the Loita Maasai of Kenya. *Environment, Development and Sustainability*, *22(3),* 2569-2589.

National Coordinating Agency for Population and Development (2008). Seeking Solutions for Traditional Herbal Medicine: Kenya Develops a National Policy. In Policy Brief No. 1.

Ndegwa, F. K. (2008). *Use, Conservation and Harvesting of Medicinal Plants by the Ogiek of East Mau Forest, Nakuru District, Kenya* (Masters Dissertation, Kenyatta University). http://thesisbank.jhia.ac.ke/id/eprint/3501

Ndhlovu, P. T., Mooki, O., Mbeng, W. O. and Aremu, A. O. (2019). Plant species used for cosmetic and cosmeceutical purposes by the Vhavenda women in Vhembe District Municipality, Limpopo, South Africa. *South African Journal of Botany, 122,* 422-431

Newman, D J. and Cragg G. M. (2016). "Natural products as sources of new drugs from 1981 to 2014." *Journal of Natural Products 79 (3),* 629-661.

Newman, D. J. and Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products, 83(3),* 770-803.

Newmaster, S. G., Grguric, M., Shanmughanandhan, D., Ramalingam, S. and Ragupathy, S. (2013). DNA barcoding detects contamination and substitution in North American herbal products. *BMC Medicine, 11(1),* 222. *https://doi.org/10.1186/1741-7015-11-222*

Newmaster, S., Ragupathy, S. and Kress, W. J. (2020). Authentication of medicinal plant components in North America's NHP industry using molecular diagnostic tools. *In Máthé Á. (eds) Medicinal and Aromatic Plants of North America.* Springer, Cham, pp 325-339.

Njoroge, J. (2002). Kenya launches herbal medicine processing plant. *Science Development Network, 10* (04).

Ochwang'i, D. O., Kimwele, C. N., Oduma, J. A., Gathumbi, P. K., Mbaria, J. M. and Kiama, S. G. (2014). Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. *Journal of Ethnopharmacology, 151(3),* 1040-1055.

Okello, S. V., Nyunja, R. O., Netondo, G. W. and Onyango, J. C. (2010). Ethnobotanical study of medicinal plants used by Sabaots of Mt. Elgon Kenya. *African Journal of Traditional, Complementary and Alternative Medicines, 7(1),* 1-10.

Okoth, P., John, M., Dennis, O., Mustafa, B. and Paul, A. (2018). Molecular Footprint of Kenya's Gene Bank Repositories Based on the cp-Genome Signatures. *American Journal of Molecular Biology, 8(4),* 215-244.

Okumu, M. O., Ochola, F. O., Onyango, A. O., Mbaria, J. M., Gakuya, D. W., Kanja, L. W., Kiama, S.G and Onyango, M. A. (2017). The legislative and regulatory framework governing herbal medicine use and practice in Kenya: a review. *Pan African Medical Journal, 28. https://doi.org/10.11604/pamj.2017.28.232.12585*

Palhares, R. M., Baratto, L. C., Scopel, M., Mügge, F. and Brandão, M. G. (2021). Medicinal Plants and Herbal Products From Brazil: How Can We Improve Quality?. *Frontiers in Pharmacology*, *11*, 2412. *https://doi.org/10.3389/fphar.2020.606623*

Palhares, R. M., Drummond, M. G., dos Santos Alves F. B. B., Cosenza, G. P., das Graças Lins B. M. and Oliveira, G. (2015). Medicinal plants recommended by the World Health Organization: DNA barcode identification associated with chemical analyses guarantees their quality. *PLOS ONE, 10(5),* e0127866. *https://doi.org/10.1371/journal.pone.0127866*

Pan, S. Y., Litscher, G., Gao, S. H., Zhou, S. F., Yu, Z. L., Chen, H. Q., Zhang, S.F., Tang, M.K., Sun, J.N. and Ko, K. M. (2014). Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evidence-based complementary and alternative medicine*, *2014*.

Pang, X. and Chen, S. (2019). Identification of Medicinal Plants Using DNA Barcoding Technique. In Encyclopedia of Analytical Chemistry *6*, 1–5. https://doi.org/10.1002/9780470027318.a9935.pub2

Peltzer, K. and Pengpid, S. (2019). The use of herbal medicines among chronic disease patients in Thailand: a cross-sectional survey. *Journal of Multidisciplinary Healthcare*, *12*, 573

Phillips, O. and Gentry, A. H. (1993). The useful plants of Tambopata, Peru: I. Statistical hypotheses tests with a new quantitative technique. *Economic Botany, 47(1),* 15–32. *https://doi.org/10.1007/BF02862203*

Qato, M. D., Alexander, G. C., Conti, R. M., Johnson, M., Schumm, Te, S. and Lindau, S. (2008). Use of prescription and over-the-counter medications and dietary supplements among older adults in the United States. *JAMA, 300(24),* 2867. *https://doi.org/10.1001/jama.2008.892*

Quail, M. A., Smith, M., Coupland, P., Otto, T. D., Harris, S. R., Connor, T. R., Bertoni, A., Swerdlow, H.P. and Gu, Y. (2012). A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics, 13(1),* 1-13.

Quiroz, D., Towns, A., Legba, S. I., Swier, J., Brière, S., Sosef, M. and van Andel, T. (2014). Quantifying the domestic market in herbal medicine in Benin, West Africa. *Journal of Ethnopharmacology, 151(3),* 1100-1108.

Raclariu, A. C., Mocan, A., Popa, M. O., Vlase, L., Ichim, M. C., Crisan, G., Brysting A. K. and de Boer, H. (2017). Veronica officinalis product authentication using DNA metabarcoding and HPLC-MS reveals widespread adulteration with Veronica chamaedrys. *Frontiers in Pharmacology, 8,* 378. *https://doi.org/10.3389/fphar.2017.00378*

Ragupathy, S., Faller, A. C., Shanmughanandhan, D., Kesanakurti, P., Shaanker, R. U., Ravikanth, G., Sathishkumar, R., Mathivanan, N., Song, J., Han, J. and Newmaster, S. (2019). Exploring DNA quantity and quality from raw materials to botanical extracts. *Heliyon, 5(6), e01935.*

Rehman, M. N., Ahmad, M., Sultana, S., Zafar, M. and Edwards, S. (2017). Relative popularity level of medicinal plants in Talagang, Punjab Province, Pakistan. *Revista Brasileira de Farmacognosia, 27(6),* 751-775.

Ribnicky, D. M., Poulev, A., Schmidt, B., Cefalu, W. T. and Raskin, I. (2008). Evaluation of botanicals for improving human health. *American Journal of Clinical Nutrition, 87(2),* 472–475. *https://doi.org/10.1093/ajcn/87.2.472s*

Rognes, T., Flouri, T., Nichols, B., Quince, C. and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ, 4,* e2584.

Rohland, N. and Hofreiter, M. (2007). Ancient DNA extraction from bones and teeth. *Nature Protocols, 2(7),* 1756–1762

Ryberg, M. (2015). Molecular operational taxonomic units as approximations of species in the light of evolutionary models and empirical data from Fungi. *Molecular Ecology, 24(23),* 5770–5777. *https://doi.org/10.1111/mec.13444*

Samarakoon, T., Wang, S. Y. and Alford, M. H. (2013). Enhancing PCR amplification of DNA from recalcitrant plant specimens using a trehalose-based additive. *Applications in Plant Sciences, 1(1), 1200236. https://doi.org/10.3732/apps.1200236*

Schmieder, R. and Edwards, R. (2011). Quality control and preprocessing of metagenomic datasets. *Bioinformatics, 27(6),* 863–864. *https://doi.org/10.1093/bioinformatics/btr026*

Seethapathy, G. S., Raclariu-Manolica, A.-C. C., Anmarkrud, J. A., Wangensteen, H. and de Boer, H. J. (2019). DNA metabarcoding authentication of ayurvedic herbal products on the European market raises concerns of quality and fidelity. *Frontiers in Plant Science, 10, 68. https://doi.org/10.3389/fpls.2019.00068*

Sgamma, T., Lockie-Williams, C., Kreuzer, M., Williams, S., Scheyhing, U., Koch, E., Slater, A. and Howard, C. (2017). DNA barcoding for industrial quality assurance. *Planta Medica, 83(14/15*), 1117-1129. *https://doi.org/10.1055/s-0043-113448*

Shanmughanandhan, D., Ragupathy, S., Newmaster, S. G., Mohanasundaram, S. and Sathishkumar, R. (2016). Estimating herbal product authentication and adulteration in India using a vouchered, DNA-based biological reference material library. *Drug Safety, 39(12),* 1211-1227. *https://doi.org/10.1007/s40264-016-0459-0*

Sheidai, M., Tabaripour, R., Talebi, S. M., Noormohammadi, Z. and Koohdar, F. (2019). Adulteration in medicinally important plant species of Ziziphora in Iran

market: DNA barcoding approach. *Industrial Crops and Products, 130,* 627–633. *https://doi.org/10.1016/j.indcrop.2019.01.025*

Sheth, B. P. and Thaker, V. S. (2015). Identification of a herbal powder by deoxyribonucleic acid barcoding and structural analyses. *Pharmacognosy Magazine, 11(Suppl 4),* S570.

Shinwari, Z. K. (2010). Medicinal plants research in Pakistan. *Journal of Medicinal Plants Research, 4(3),* 161-176.

Sifuna, N. (2022). Configuring a model framework statute on Traditional Medicine for Kenya: To Be Called "The Traditional Medicine Coordination Act*". Beijing Law Review,* 13(1), 183-203.

Silambarasan, R., Sureshkumar, J., Krupa, J., Amalraj, S. and Ayyanar, M. (2017). Traditional herbal medicines practiced by the ethnic people in Sathyamangalam forests of Western Ghats, India. *European Journal of Integrative Medicine, 16,* 61-72.

Srirama, R., Santhosh Kumar, J. U., Seethapathy, G. S., Newmaster, S. G., Ragupathy, S., Ganeshaiah, K. N., Uma Shaanker, R. and Ravikanth, G. (2017). Species adulteration in the herbal trade: causes, consequences and mitigation. *Drug safety, 40(8),* 651-661.

Srirama, R., Senthilkumar, U., Sreejayan, N., Ravikanth, G., Gurumurthy, B. R., Shivanna, M. B., Sanjappa, M., Ganeshaiah, K.N. and Shaanker, R. U. (2010). Assessing species admixtures in raw drug trade of Phyllanthus, a hepato-protective plant using molecular tools. *Journal of Ethnopharmacology, 130(2),* 208–215. *https://doi.org/10.1016/J.JEP.2010.04.042*

Staats, M., Arulandhu, A. J., Gravendeel, B., Holst-Jensen, A., Scholtens, I., Peelen, T., Prins, T.W. and Kok, E. (2016). Advances in DNA metabarcoding for food and wildlife forensic species identification. *Analytical and Bioanalytical Chemistry, 408(17),* 4615–4630. *https://doi.org/10.1007/s00216-016-9595-8*

Stoeckle, M. Y., Gamble, C. C., Kirpekar, R., Young, G., Ahmed, S. and Little, D. P. (2011). Commercial teas highlight plant DNA barcode identification successes and obstacles. *Scientific Reports, 1(1),* 42. https://doi.org/10.1038/srep00042

Sui, X., Huang, Y., Tan, Y., Guo, Y. and Long, C. (2011). Molecular authentication of the ethnomedicinal plant *sabia parviflora* and its adulterants by DNA barcoding technique. *Planta Medica, 77(05),* 492–496. *https://doi.org/10.1055/s-0030-1250468*

Sun, Y., Skinner, D. Z., Liang, G. H. and Hulbert, S. H. (1994). Phylogenetic analysis of sorghum and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics, 89(1),* 26–32. *https://doi.org/10.1007/BF00226978*

Swiderska, K. (2006). Protecting community rights over traditional knowledge: implications of customary laws and practices. International Institute for Environment and Development. Available online (http://hdl.handle.net/10625/28678). Accessed 2 nd July 2019.

Techen, N., Crocketnatat, S., Khan, I. and Scheffler, B. (2012). Authentication of medicinal plants using molecular biology techniques to compliment conventional methods. *Current Medicinal Chemistry, 11(11),* 1391–1401. *https://doi.org/10.2174/0929867043365206*

Techen, N., Parveen, I., Pan, Z. and Khan, I. A. (2014). DNA barcoding of medicinal plant material for identification. *Current Opinion in Biotechnology, 25,* 103-110.

Tefera, B. N. and Kim, Y. D. (2019). Ethnobotanical study of medicinal plants in the Hawassa Zuria District, Sidama zone, Southern Ethiopia. *Journal of Ethnobiology and Ethnomedicine, 15(1),* 1-21.

The partnership for safe medicine (PSM). (2010). Kenya Creates New Regulations for Herbal Remedies. Available online (https://www.safemedicines.org/2010/11/kenya-creates-new-regulations-for-herbal-remedies-14.html). Accessed on 2nd August 2019.

Tnah, L. H., Lee, S. L., Tan, A. L., Lee, C. T., Ng, K. K. S., Ng, C. H. and Nurul, F. Z. (2019). DNA barcode database of common herbal plants in the tropics: a resource for herbal product authentication. *Food Control, 95,* 318–326. *https://doi.org/10.1016/j.foodcont.2018.08.022*

Tsumura, Y., Yoshimura, K., Tomaru, N. and Ohba, K. (1995). Molecular phytogeny of conifers using RFLP analysis of PCR-amplified specific chloroplast genes. *Theoretical and Applied Genetics, 91(8),* 1222-1236.

Urumarudappa, S. K. J., Tungphatthong, C., Prombutara, P. and Sukrong, S. (2020). DNA metabarcoding to unravel plant species composition in selected herbal medicines on the National List of Essential Medicines (NLEM) of Thailand. *Scientific Reports, 10(1),* 1-11.

Urumarudappa, S. K. J., Gogna, N., Newmaster, S. G., Venkatarangaiah, K., Subramanyam, R., Saroja, S. G., Gudasalamani, R., Dorai, K. and Ramanan, U.S. (2016). DNA barcoding and NMR spectroscopy-based assessment of species adulteration in the raw herbal trade of *Saraca asoca* (Roxb.) Willd, an important medicinal plant. *International Journal of Legal Medicine, 130(6),* 1457-1470. *https://doi.org/10.1007/s00414-016-1436-y*

Veldman, S., Gravendeel, B., Otieno, J. N., Lammers, Y., Duijm, E., Nieman, A., Bytebier, B., Ngugi, G., Martos, F., van Andel, T.R. and de Boer, H. J. (2017). High-throughput sequencing of African chikanda cake highlights

conservation challenges in orchids. *Biodiversity and Conservation, 26,* 2029–2046. *https://doi.org/10.1007/s10531-017-1343-7*

Veldman, S., Ju, Y., Otieno, J. N., Abihudi, S., Posthouwer, C., Gravendeel, B., van Andel, T.R. and de Boer, H. J. (2020). DNA barcoding augments conventional methods for identification of medicinal plant species traded at Tanzanian markets. *Journal of Ethnopharmacology*, *250*, 112495. *https://doi.org/10.1016/j.jep.2019.112495*

Venkatachalapathi, A., Sangeeth, T., Ali, M. A., Tamilselvi, S. S., Paulsamy, S. and Al-Hemaidc, F. M. (2018). Ethnomedicinal assessment of Irula tribes of Walayar valley of Southern Western Ghats, India. *Saudi Journal of Biological Sciences, 25(4),* 760-775.

Wachtel-Galor S. and Benzie I.F.F. (2011) Herbal Medicine: An Introduction to Its History, Usage, Regulation, Current Trends, and Research Needs. In: Benzie IFF, Wachtel-Galor S, (eds). Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor and Francis; Chapter 1. PMID: 22593939 Available online (https://www.ncbi.nlm.nih.gov/books/NBK92773/). Accessed 20th November 2018.

Wallace, L. J., Boilard, S. M. A. L. A. L., Eagle, S. H. C. C., Spall, J. L., Shokralla, S. and Hajibabaei, M. (2012). DNA barcodes for everyday life: Routine authentication of Natural Health Products. *Food Research International, 49 (1)* 446-452 *https://doi.org/10.1016/j.foodres.2012.07.048*

Wei, W., Youliang, Z., Li, C., Yuming, W., Zehong, Y. and Ruiwu, Y. (2005). PCR-RFLP analysis of cpDNA and mtDNA in the genus *Houttuynia* in some areas of China. *Hereditas, 142,* 24-32.

Welz, A. N., Emberger-Klein, A. and Menrad, K. (2018). Why people use herbal medicine: Insights from a focus-group study in Germany. *BMC Complementary and Alternative Medicine, 18(1), 1–9. https://doi.org/10.1186/s12906-018-2160-6*

World Health Organisation (WHO) (2011). The World Medicines Situation 2011 Traditional Medicines : Global Situation, Issues and Challenges. World Health Organization, (3rd Edition) Available online (*http://digicollection.org/hss/documents/s18063en/s18063en.pdf).* Accessed 2nd August 2019

World Health Organization (WHO) (2000). General guidelines for methodologies on research and evaluation of traditional medicine (No. WHO/EDM/TRM/2000.1). World Health Organization.

World Health Organization (WHO) (2005). National policy on traditional medicine and regulation of herbal medicines: Report of a WHO global survey. World Health Organization

Xin, T., Li, X., Yao, H., Lin, Y., Ma, X., Cheng, R., Song, J., Ni, L., Fan, C. and Chen, S. (2015). Survey of commercial Rhodiola products revealed species diversity and potential safety issues. *Scientific Reports, 5(1),* 8337. *https://doi.org/10.1038/srep08337*

Xin, T., Xu, Z., Jia, J., Leon, C., Hu, S., Lin, Y., Ragupathy, S., Song, J. and Newmaster, S. G. (2018). Biomonitoring for traditional herbal medicinal products using DNA metabarcoding and single molecule, real-time sequencing. *Acta Pharmaceutica Sinica B, 8(3),* 488–497. *https://doi.org/10.1016/j.apsb.2017.10.001*

Xue, C. Y. and LI, D. Z. (2011). Use of DNA barcode sensu lato to identify traditional Tibetan medicinal plant *Gentianopsis paludosa* (Gentianaceae). *Journal of Systematics and Evolution, 49(3),* 267-270.

Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y. and Pang, X. (2010). Use of ITS2 region as the universal DNA barcode for plants and animals. *PLoS One, 5,* e13102

Yu, M., Liu, K., Zhou, L., Zhao, L. and Liu, S. (2016). Testing three proposed DNA barcodes for the wood identification of *Dalbergia odorifera* T. Chen and *Dalbergia tonkinensis* Prain. *Holzforschung, 70(2). https://doi.org/10.1515/hf-2014-0234*

Yu, N., Wei, Y. L., Zhu, Y., Zhu, N., Wang, Y. L., Zhang, H. P. and Sun, A. D. (2018). Integrated approach for identifying and evaluating the quality of Marsdenia tenacissima in the medicine market. *PLoS One, 13*(4), e0195240.

Zhang, D. and Jiang, B. (2020). Species identification in complex groups of medicinal plants based on DNA barcoding: a case study on *Astragalus spp. (*Fabaceae) from Southwest China. *Conservation Genetics Resources, 12(3),* 469-478.

Zhang, P., Liu, C., Zheng, X., Wu, L., Liu, Z., Liao, B., Shi, Y., Li, X., Xu, J. and Chen, S. (2019). Full-length multi-barcoding: DNA barcoding from single species to complex mixtures. *Genes*, *10*(5), 343.

Zhao, L. L., Feng, S. J., Tian, J. Y., Wei, A. Z. and Yang, T. X. (2018). Internal transcribed spacer 2 (ITS 2) barcodes: A useful tool for identifying Chinese Zanthoxylum. *Applications in plant sciences, 6(6),* e01157.

APPENDICES

Appendix I: Questionnaire for Market Survey of Herbal Medicine Trade in Kajiado, Nairobi and Narok Counties

Introduction

I am Ann Mwaura, a PhD student at Kenyatta University and affiliated to National Museums of Kenya. Am carrying out a study on; 'Identification of commonly used medicinal plant species in Kenya using DNA barcoding'. Part of my data collection involves carrying out an ethnobotanical market survey to establish plant species of medicinal value that are being traded in Nairobi, Kajiado and Narok regions. All information provided will be treated confidential and used for research only.

Site data

Date		Serial No	
City / Town			
Market			
Sub-County			
County			

a. Biodata

Name (optional)	
Age	
Sex	
Religion	
Tribe (community)	
Main occupation Collector/supplier	
Education	

b. Main consumers/customers for the herbal products

Gender	0-20 years	21-30 years	31-40 years	41-50 years	>51years
Males					
Females					

c. Trade and threat to plant/ plant materials traded

1. How long (years) have you been in this business of herbal products…0-1( ) 1-3 ( ) 4-6 ( ) >6 years ( )
2. What motivated you to start this business of herbal products?
   …….....

3. Why do you think people prefer these herbal products?.....(choose from the list below)
a. They are cheap ( ) b. They are easily accessible ( ) c. They are more effective ( ) d. They are more affordable ( ) e. People lack alternatives ( ) f. They are more durable ( ) g. Aesthetics h. Other reasons (specify)…….,.....
4. How many different plant/herbal products are you selling?
5. How do you get the plant products (choose from below)
a. I collect them myself from the wild
b. I buy from middle men
c. Buy from stalls in town
d. From an established facility/farm outlet
e. I import from outside
6. If 5a above applies, what are the challenges you face in collecting your plants/plant products?
a. Availability
b. Distance
c. Legislation/licenses
d. Others challenges (specify)……..
7. What are some of the challenges associated in buying from middle men?
a. High costs
b. Delayed/unreliable delivery
c. Unknown source
d. Adulteration
e. Others challenges (specify)……..
8. Which areas are known for supplying most of plants/plant products you are selling?
……..
9. Do any of these areas require permits? Yes ( ) …No ( )…. If yes, please mention these areas
……..
a. How many times in a week/month/year do you procure your stock?
……...
b. How much do you take at a time?
…….
10. Estimate the average purchase price of the plant/plant product per kg……
11. How much do you have in storage right now?
.....……
12. Do you sell your plants/plant products outside Kenya?
a. Yes b. No
13. Which countries are the most important foreign markets for your plant/plant products?.....
...
14. Do you know any plant/plant products that are sold outside Kenya by other people? List them and their markets
1…… 2.....

3..... 4.....

15. Name the 5 plants/plant products that are commonly traded in this market?

1……...2.....3.....

.....

4.....5……6……

……

16. Why in your opinion are they popular/ or their demand high? (each plant/product)

……

……

……

……

…..….

17. Do you think the high demand affects these plants in the wild? Yes ( ) No ( )
If yes, how?

……..……

……..……

18. Are there plants/plant products that were previously traded but are no longer in the supply? Please list them

…….

…….

For how long now has this been the case?

……

…..……

……

……

19. What in your opinion has caused the decline in supply of the plant/plant products?

.....

.....

.....

20. Please estimate the amount of money in Kshs. you earn from trading in these plants/plant products per month?

a) < 5000
b) 5001-10,000
c) 10,001-20,000
d) 20,001-50,000
e) >50,000

21. Please provide us with the list of plants/plant products that you are trading and other information as 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	Where collected/ obtained	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	Method of preparation

I have full consent to give Ann Mwaura information as it relates to the plants and plant materials in my possession for purpose of this survey.

Signed----------(Interviewee) Signed .....(Interviewer)

Date ---------- (*dd/mm/yy*) Date ..... (*dd/mm/yy*)

Appendix II: Group discussion and sampling of herbal products in Ngong market, Kajiado County

Appendix III: Sampling of reference voucher specimens by student and taxonomist in Melili, Narok County

Osyris lanceolata (Sandal wood): leading species recorded during market survey

Appendix IV: Summary list of herbal plant species generated from mixed species samples (pages 132 to 134)

S. no	Species names indicated on the sample label	Family	Common names⁑
1	*Acacia kirkii* Oliv.	Fabaceae[1]	
2	*Acacia mellifera* (M.Vahl) Benth.	Fabaceae[1]	
3	*Ajuga remota* Benth.	Lamiaceae[9]	
4	*Albizia anthelmintica* Brongn	Fabaceae[1]	
5	*Aloe secundiflora* Engl.	Xanthorrhoeaceae[2]	
6	*Aloe vera* (L.) Burm.f.	Xanthorrhoeaceae[2]	
7	*Amaranthus dubius* Mart.	Amaranthaceae [3]	
8	*Annona muricata* L.	Annonaceae[4]	Graviola fruit powder
9	*Azadirachta indica* A.Juss.	Meliaceae[5]	Neem bark
10	*Carica papaya* L.	Caricaceae[6]	
11	*Carissa edulis* Forssk.	Apocynaceae[7]	
12	*Cassia abbreviata* Oliv.	Fabaceae [1]	
13	*Catharanthus roseus* (L.) G.Don	Apocynaceae[7]	Periwinkal leaves
14	*Clausena anisata* (Willd.) Hook.f. ex Benth.	Rutaceae[8]	
15	*Clerodendrum heterophyllum* (Poir.) R.Br.	Lamiaceae[9]	
16	*Croton megalocarpus* Hutch	Euphorbiaceae [10]	
17	*Croton dichogamus* Pax	Euphorbiaceae[10]	
18	*Cucurbita pepo* L.	Cucurbitaceae[12]	Pumpkin seeds
19	*Cymbopogon citratus* (DC.) Stapf	Poaceae[39]	Lemon grass
20	*Delonix elata* (L.) Gamble	Fabaceae[1]	
21	*Dombeya rotundifolia* (Hochst.) Planch.	Malvaceae[13]	
22	*Euclea divinorum* Hiern	Ebenaceae[14]	
23	*Faurea saligna* Harv.	Proteaceae[15]	
24	*Fuerstia africana* T.C.E.Fr.	Lamiaceae[9]	
25	*Grewia similis* K.Schum	Malvaceae[13]	
26	*Heuchera micrantha* Douglas ex Lindl	Saxifragaceae[17]	Alum/ alam roots
27	*Hibiscus flavifolius* Ulbr.	Malvaceae[13]	Hibiscus
28	*Hypericum perforatum* L.	Hypericaceae[18]	John wort
29	*Lantana camara* L.	Verbenaceae[19]	
30	*Linum usitatissimum* L.	Linaceae[20]	Linseeds/Flax seeds
31	Mentha (mint)	Lamiaceae[9]	Mint
32	*Moringa oleifera* Lam.	Moringaceae	
33	*Myrsine africana* L.	Primulaceae	

S. no	Species names indicated on the sample label	Family	Common names⁑
34	*Ocimum gratissimum* L.	Lamiaceae[9]	
35	*Olea capensis* L.	Oleaceae[21]	
36	*Olea europaea* L.	Oleaceae[21]	
37	*Osyris lanceolata* Hochst. & Steud.	Santalaceae[22]	
38	*Pappea capensis* Eckl. & Zeyh.	Sapindaceae[23]	
39	*Pennisetum glaucum* (L.) R.Br.	Poaceae[39]	
40	*Phyllanthus emblica* L.	Phyllanthaceae[24]	Indian goosebery
41	*Pinus spp* (pine bark)	Pinaceae[25]	
42	*Pittosporum mannii* Hook.f.	Pittosporaceae[26]	
43	*Plumbago zeylanica* L.	Plumbaginaceae[27]	
44	*Prunus africana* (Hook.f.) Kalkman	Rosaceae[28]	
45	*Psiadia punctulata* (DC.) Vatke	Acanthaceae[11]	
46	*Rhamnus prinoides* L'Hér.	Rhamnaceae[29]	
47	*Rhamnus staddo* A.Rich.	Rhamnaceae[29]	
48	*Rhus natalensis* Bernh. ex C.Krauss	Anacardiaceae[30]	
49	*Rosmarinus officinalis* L	Lamiaceae[9]	Rosemary
50	*Rotheca myricoides* (Hochst.) Steane & Mabb.	Lamiaceae[9]	
51	*Rumex crispus* L.	Polygonaceae[31]	Yellow dock
52	*Salvadora persica* L.	Salvadoraceae[32]	
53	*Schinus molle* L.	Anacardiaceae[30]	Pepper tree seeds
54	*Senna italica* Mill.	Fabaceae[1]	
55	*Sesamum indicum* L.	Pedaliaceae[34]	
56	*Stellaria media* (L.) Vill.	Caryophyllaceae[35]	Chicken weed
57	*Strychnos henningsii* Gilg	Loganiaceae[37]	
58	*Taraxacum officinale* (L.) Weber ex F.H.Wigg. (*Taraxacum campylodes* G.E.Haglund)	Compositae[38]	Dandelion
59	*Teclea simplicifolia* (Engl.) I. Verd. (*Vepris simplicifolia* (Engl.) Mziray)	Rutaceae[8]	
60	*Toddalia asiatica* (L.) Lam.	Rutaceae[8]	
61	*Trigonella foenum-graecum* L	Fabaceae[1]	Fenugreek
62	*Triticum aestivum* L.	Poaceae[39]	Wheatgrass
63	*Turraea abyssinica* Hochst.	Meliaceae[5]	
64	*Urtica massaica* Mildbr.	Urticaceae[41]	
65	*Urtica dioica* L.	Urticaceae[41]	Stinging nettle
66	*Vangueria infausta* Burch.	Rubiaceae[43]	
67	*Vernonia brachycalyx* O.Hoffm.	Compositae[38]	
68	*Warburgia ugandensis* Sprague	Canellaceae[40]	

S. no	Species names indicated on the sample label	Family	Common names‡
69	*Withania somnifera* (L.) Dunal	Solanaceae[33]	
70	*Ximenia americana* L.	Olacaceae[16]	
71	*Zanthoxylum chalybeum* Engl.	Rutaceae[8]	
72	*Zanthoxylum usambarense* (Engl.) Kokwaro	Rutaceae[8]	
73	*Zehneria scabra* Sond.	Cucurbitaceae[12]	

Key: ‡ Common name as given on the label. Blank, the respondent did not indicate the name on the label. Number in superscript represent the family count of the 73 species (N=43).

Appendix V: Mixed species samples analyzed using metabarcoding (pages 135 to 163: complete Table 4.5)

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
1	MAN2	2	*Azadirachta indica* A.Juss. (neem) *Moringa oleifera* Lam.	*Moringa oleifera* Lam. *Amaranthus dubius* Mart. *Capsicum annuum* L. *Cassia sp.* *Duranta erecta* L. *Gynandropsis gynandra* (L.) Briq. / *Cleome gynandra* L. *Ipomoea batatas* (L.) Lam. *Pittosporum mannii* Hook.f. *Salvia hispanica* L. *Senna italica* Mill. *Senna montana* (Roth) V.Singh *Senna occidentalis* (L.) Link *Senna siamea* (Lam.) H.S.Irwin & Barneby *Sesamum indicum* L. *Solanum lycopersicum* L. *Sorghum bicolor* (L.) Moench *Urtica dioica* L. *Urtica sp.* *Zea mays* L.	Only 1 species on label was detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
2	MAN4	2	*Moringa oleifera* Lam. *Azadirachta indica* A.Juss. (neem)	*Moringa oleifera* Lam. *Albizia anthelmintica* Brongn *Amaranthus dubius* Mart. *Grevillea robusta* A.Cunn. ex R.Br. *Myristica fragrans* Houtt. *Nicotiana tabacum* L. *Secale cereale* L. *Urtica dioica* L. *Zea mays* L.	Only 1 species on label was detected
3	MAN5		*Triticum aestivum* L. (wheatgrass) *Urtica dioica* L.	*Triticum aestivum* L. *Urtica dioica* L. *Aegilops biuncialis* Vis. *Aegilops cylindrica* Host *Digitaria velutina* (Forssk.) P.Beauv. *Hordeum vulgare* L. *Secale cereale* L. *Triticum aestivum* L. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. *Triticum monococcum* L. *Triticum turgidum* L. *Urtica dioica* L.	Only 2 species on label were detected
4	MAN6	6	*Ajuga remota* Benth.	*Ajuga remota* Benth.	Only 5 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Croton dichogamus Pax *Moringa oleifera* Lam. *Urtica dioica* L. *Withania somnifera* (L.) Dunal *Zehneria scabra* Sond.	*Croton dichogamus* Pax *Urtica dioica* L. *Withania somnifera* (L.) Dunal *Zehneria scabra* Sond. *Amaranthus dubius* Mart. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Balanites glabra* Mildbr. & Schltr. *Bidens pilosa* L. *Cucurbita pepo* L. *Cynodon aethiopicus* Clayton & Harlan *Digitaria velutina* (Forssk.) P.Beauv. *Galinsoga parviflora* Cav. *Galinsoga quadriradiata* Ruiz & Pav. *Ocimum basilicum* L. *Ocimum gratissimum* L. *Oryza sativa* L. *Pennisetum glaucum* (L.) R.Br. *Rumex crispus* L. *Sorghum bicolor* (L.) Moench *Triticum aestivum* L. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. *Triticum turgidum* L. *Zea mays* L.	

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
				Zehneria sp.	
5	MAN7	4	*Euclea divinorum* Hiern *Osyris lanceolata* Hochst. & Steud. *Salvadora persica* L. *Vangueria infausta* Burch.	*Euclea divinorum* Hiern *Osyris lanceolata* Hochst. & Steud. *Vangueria infausta* Burch. *Albizia anthelmintica* Brongn *Amaranthus dubius* Mart. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Ocimum gratissimum* L.	Only 3 species on label were detected
6	MAN8	3	*Albizia anthelmintica* Brongn *Azadirachta indica* A.Juss. *Osyris lanceolata* Hochst. & Steud.	*Albizia anthelmintica* Brongn *Azadirachta indica* A.Juss. *Osyris lanceolata* Hochst. & Steud. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." C*ommelina communis* *Oryza sativa* L. *Pittosporum mannii* Hook.f. *Senna italica* Mill. *Senna occidentalis* (L.) Link	Only 3 species on label were detected
7	MAN9	4	*Dombeya rotundifolia* (Hochst.) Planch. *Osyris lanceolata* Hochst. & Steud. *Rhus natalensis* Bernh. ex C.Krauss	*Dombeya rotundifolia* (Hochst.) Planch. Osyris lanceolata Hochst. & Steud. *Zanthoxylum usambarense* (Engl.) Kokwaro	Only 3 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Zanthoxylum usambarense (Engl.) Kokwaro	*Amaranthus dubius* Mart. *Zea mays* L.	
8	MAN10	3	*Amaranthus dubius* Mart. *Albizia anthelmintica* Brongn *Cassia abbreviata* Oliv.	*Albizia anthelmintica* Brongn *Cassia sp*. *Senna montana* (Roth) V.Singh *Zehneria sp*	Only 1 species on label was detected
9	MAN11	13	*Ajuga remota* Benth. *Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Carica papaya* L. *Carissa edulis* Forssk. Forssk. *Euclea divinorum* Hiern *Pappea capensis* Eckl. & Zeyh. *Salvadora persica* L. *Trigonella foenum-graecum* L *Urtica dioica* L. *Warburgia ugandensis* Sprague *Withania somnifera* (L.) Dunal *Zanthoxylum usambarense* (Engl.) Kokwaro	*Ajuga remota* Benth. *Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Carica papaya* L. *Carissa edulis* Forssk. *Euclea divinorum* Hiern *Pappea capensis* Eckl. & Zeyh. *Salvadora persica* L. *Trigonella foenum-graecum* L *Urtica dioica* L. *Warburgia ugandensis* Sprague *Withania somnifera* (L.) Dunal *Zanthoxylum usambarense* (Engl.) Kokwaro *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord."	All species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
				Coriandrum sativum L. *Croton dichogamus* Pax *Cuminum cyminum* L. *Dombeya rotundifolia* (Hochst.) Planch. *Galinsoga parviflora* Cav. *Hordeum vulgare* L. *Ocimum americanum L.* *Ocimum basilicum* L. *Oryza sativa* L. *Osyris lanceolata* Hochst. & Steud. *Pittosporum mannii* Hook.f. R*umex spinosus L.* *Secale cereale* L. *Senna italica* Mill. *Triticum aestivum* L.	
10	MAN13	3	*Rumex crispus* L. (Yellow dock) *Salvadora persica* L. *Azadirachta indica* A.Juss.	*Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Balanites glabra* Mildbr. & Schltr. *Ipomoea batatas* (L.) Lam. *Salvadora persica* L.	No species on label detected
11	MAN17	10	*Aloe vera* (L.) Burm.f. *Amaranthus dubius* Mart. *Clerodendrum heterophyllum* (Poir.) R.Br. *Osyris lanceolata* Hochst. & Steud.	*Aloe vera* (L.) Burm.f. *Amaranthus dubius* Mart. *Clerodendrum heterophyllum* (Poir.) R.Br. *Osyris lanceolata* Hochst. & Steud.	All species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Pittosporum mannii Hook.f. *Salvadora persica* L. *Senna italica* Mill. *Vernonia brachycalyx* O.Hoffm.O. *Warburgia ugandensis* Sprague *Zanthoxylum usambarense* (Engl.) Kokwaro	*Pittosporum mannii* Hook.f. *Salvadora persica* L. *Senna italica* Mill. *Vernonia brachycalyx* O.Hoffm. *Warburgia ugandensis* Sprague *Zanthoxylum usambarense* (Engl.) Kokwaro *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Dombeya rotundifolia* (Hochst.) Planch. *Galinsoga parviflora* Cav. *Ocimum basilicum* L. *Pennisetum glaucum* (L.) R.Br. *Sesamum indicum* L. *Sorghum bicolor* (L.) Moench *Triticum aestivum* L. *Zea mays* L.	
12	MAN19	6	*Albizia anthelmintica* Brongn *Clerodendrum heterophyllum* (Poir.) R.Br. *Pittosporum mannii* Hook.f. *Prunus africana* (Hook.f.) Kalkman *Senna italica* Mill.	*Albizia anthelmintica* Brongn *Clerodendrum heterophyllum* (Poir.) R.Br. *Pittosporum mannii* Hook.f. *Senna italica* Mill. *Warburgia ugandensis* Sprague	Only 5 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Warburgia ugandensis Sprague	*Anacardiaceae sp.* *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Balanites glabra* Mildbr. & Schltr. *Capsicum annuum* L. *Hordeum vulgare* L. *Ocimum basilicum* L. *Oryza sativa* L. *Pennisetum glaucum* (L.) R.Br. *Salvadora persica* L. *Secale cereale* L. *Triticum aestivum* L. *Zea mays* L.	
13	MAN22	7	*Albizia anthelmintica* Brongn *Aloe secundiflora* Engl. *Azadirachta indica* A.Juss. (Neem pellets) *Olea europaea* L. *Olea capensis* L. *Rumex crispus* L. *Warburgia ugandensis* Sprague	*Albizia anthelmintica* Brongn *Rumex crispus* L. *Warburgia ugandensis* Sprague *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Aloe vera* (L.) Burm.f. *Amaranthus dubius* Mart. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Carica papaya* L. *Clausena anisata* (Willd.) Hook.f. ex Benth.	Only 1 species on label was detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
				Croton dichogamus Pax *Croton megalocarpus* Hutch *Pennisetum glaucum* (L.) R.Br. *Piper sp*. *Pittosporum mannii* Hook.f. *Secale cereale* L. *Senna italica* Mill. *Sorghum bicolor* (L.) Moench *Triticum aestivum* L. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. *Urtica dioica* L. *Zea mays* L.	
13	MAN23	3	*Moringa oleifera* Lam. *Warburgia ugandensis* Sprague *Salvadora persica* L.	*Warburgia ugandensis* Sprague *Salvadora persica* L. *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Aloe vera* (L.) Burm.f. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Croton megalocarpus* Hutch *Cuminum cyminum* L. *Ocimum basilicum* L. *Oryza sativa* L. *Rosmarinus officinalis* L	Only 2 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
				Secale cereale L. *Senna montana* (Roth) V.Singh *Triticum aestivum* L. *Triticum turgidum* L. *Urtica dioica* L. *Zea mays* L.	
15	MAN24	3	*Carica papaya* L. *Pappea capensis* Eckl. & Zeyh. *Urtica massaica* Mildbr.	*Urtica massaica* Mildbr. *Cucurbita pepo* L. *Cynodon aethiopicus* Clayton & Harlan *Galinsoga parviflora* Cav. *Solanum tuberosum* L. *Stellaria media* (L.) Vill. *Thunbergia alata* Bojer ex Sims *Zea mays* L.	Only 1 species on label was detected
16	MAN25	7	*Ajuga remota* Benth. *Albizia anthelmintica* Brongn *Clerodendrum heterophyllum* (Poir.) R.Br. *Osyris lanceolata* Hochst. & Steud. *Pittosporum mannii* Hook.f. *Rhamnus prinoides* L'Hér. *Senna italica* Mill.	*Albizia anthelmintica* Brongn *Clerodendrum heterophyllum* (Poir.) R.Br. *Osyris lanceolata* Hochst. & Steud. *Pittosporum mannii* Hook.f. *Rhamnus prinoides* L'Hér. *Senna italica* Mill. *Amaranthus dubius* Mart. *Cassia sp*.	Only 6 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
				Pennisetum glaucum (L.) R.Br. *Rhamnus staddo* A.Rich. *Urtica dioica* L. *Warburgia ugandensis* Sprague *Zanthoxylum sp.* *Zea mays* L.	
17	MAN26	3	*Amaranthus dubius* Mart. *Osyris lanceolata* Hochst. & Steud. *Urtica massaica* Mildbr.	*Amaranthus dubius* Mart. *Urtica dioica* L. *Bidens pilosa* L. *Cynodon aethiopicus* Clayton & Harlan	Only 2 species on label were detected
18	MAN27	4	*Albizia anthelmintica* Brongn *Croton dichogamus* Pax *Psiadia punctulata* (DC.) Vatke *Warburgia ugandensis* Sprague	*Albizia anthelmintica* Brongn *Croton dichogamus* Pax *Warburgia ugandensis* Sprague *Ajuga remota* Benth. *Cymbopogon citratus* (DC.) Stapf *Triticum aestivum* L. *Triticum turgidum* L. *Zea mays* L.	Only 3 species on label were detected
19	MAN28	8	*Albizia anthelmintica* Brongn *Faurea saligna* Harv. *Plumbago zeylanica* L. *Rhamnus prinoides* L'Hér.	*Albizia anthelmintica* Brongn *Faurea saligna* Harv. *Rhamnus prinoides* L'Hér. *Rotheca myricoides* (Hochst.) Steane & Mabb.	Only 7 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Rotheca myricoides (Hochst.) Steane & Mabb. *Salvadora persica* L. *Schinus molle* L. *Warburgia ugandensis* Sprague	*Salvadora persica* L. *Schinus molle* L. *Warburgia ugandensis* Sprague *Ajuga remota* Benth. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Bidens pilosa* L. *Croton dichogamus* Pax *Cymbopogon citratus* (DC.) Stapf *Musa acuminata* Colla *Ocimum gratissimum* L. *Pennisetum glaucum* (L.) R.Br. *Pentas micrantha* Baker *Rosmarinus officinalis* L *Solanum nigrum* L. *Solanum tuberosum* L. *Triticum aestivum* L. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. *Triticum turgidum* L. *Urtica dioica* L. *Zaleya pentandra* (L.) C.Jeffrey *Zanthoxylum sp.* *Zea mays* L.	

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
20	MAN29	3	*Pappea capensis* Eckl. & Zeyh. *Delonix elata* (L.) Gamble *Warburgia ugandensis* Sprague	*Delonix elata* (L.) Gamble *Warburgia ugandensis* Sprague *Urtica dioica* L. *Urtica sp.*	Only 2 species on label were detected
21	MAN30	8	*Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Euclea divinorum* Hiern *Myrsine africana* L. *Osyris lanceolata* Hochst. & Steud. *Rhamnus prinoides* L'Hér. *Withania somnifera* (L.) Dunal *Zanthoxylum usambarense* (Engl.) Kokwaro	*Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Euclea divinorum* Hiern *Myrsine africana* L. *Osyris lanceolata* Hochst. & Steud. *Rhamnus prinoides* L'Hér. *Withania somnifera* (L.) Dunal *Zanthoxylum sp*. *Ajuga remota* Benth. *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Carica papaya* L. *Ocimum gratissimum* L. *Pittosporum mannii* Hook.f. *Senna italica* Mill. *Zea mays* L.	Only 7species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
22	MAN31	7	*Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Croton dichogamus* Pax *Pittosporum mannii* Hook.f. *Rhamnus prinoides* L'Hér. *Strychnos henningsii* Gilg *Zanthoxylum usambarense* (Engl.) Kokwaro	*Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Croton dichogamus* Pax *Pittosporum mannii* Hook.f. *Rhamnus prinoides* L'Hér. *Strychnos henningsii* Gilg *Zanthoxylum usambarense* (Engl.) Kokwaro *Ajuga remota* Benth. *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Dombeya rotundifolia* (Hochst.) Planch. *Euclea divinorum* Hiern *Osyris lanceolata* Hochst. & Steud. *Secale cereale* L. *Senna occidentalis* (L.) Link *Sorghum bicolor* (L.) Moench *Triticum aestivum* L. *Triticum turgidum* L. *Urtica dioica* L. Ximenia americana L. *Zea mays* L.	All species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
23	MAN32	4	*Azadirachta indica* A.Juss. *Clerodendrum heterophyllum* (Poir.) R.Br. *Pittosporum mannii* Hook.f. *Salvadora persica* L.	*Clerodendrum heterophyllum* (Poir.) R.Br. *Pittosporum mannii* Hook.f. *Salvadora persica* L. *Albizia anthelmintica* Brongn	Only 3 species on label were detected
24	MAN33	5	*Albizia anthelmintica* Brongn *Amaranthus dubius* Mart. *Azadirachta indica* A.Juss. *Moringa oleifera* Lam. *Osyris lanceolata* Hochst. & Steud.	*Amaranthus dubius* Mart. *Azadirachta indica* A.Juss. *Moringa oleifera* Lam. *Osyris lanceolata* Hochst. & Steud. *Aloe vera* (L.) Burm.f. *Oryza sativa* L. *Persea americana* Mill. *Piper sp.* *Pittosporum mannii* Hook.f. *Salvia officinalis* L. *Rosmarinus officinalis* L *Senna occidentalis* (L.) Link *Triticum turgidum* L. *Urtica dioica* L. *Warburgia ugandensis* Sprague *Zea mays* L.	All species on label were detected
25	MAN34	7	*Albizia anthelmintica* Brongn *Grewia similis* K.Schum	*Albizia anthelmintica* Brongn *Grewia similis* K.Schum	Only 6 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Olea europaea L. *Osyris lanceolata* Hochst. & Steud. *Dombeya rotundifolia* (Hochst.) Planch. *Teclea simplicifolia* (Engl.) I. Verd. *Zanthoxylum usambarense* (Engl.) Kokwaro	*Olea europaea L.* *Osyris lanceolata* Hochst. & Steud. *Dombeya rotundifolia* (Hochst.) Planch. *Zanthoxylum usambarense* (Engl.) Kokwaro *Aloe vera* (L.) Burm.f. *Amaranthus dubius* Mart. *Cissus rotundifolia* Vahl *Cordia monoica* Roxb. *Cynodon dactylon* (L.) Pers. *Ocimum gratissimum* L. *Secale cereale* L. *Solanum lycopersicum* L. *Solanum melongena* L. *Solanum nigrum* L. *Triticum aestivum* L. *Urtica dioica* L. *Zaleya pentandra* (L.) C.Jeffrey *Zea mays* L.	
26	MAN35	7	*Albizia anthelmintica* Brongn *Croton megalocarpus* Hutch *Pittosporum mannii* Hook.f. *Salvadora persica* L.	*Albizia anthelmintica* Brongn *Croton megalocarpus* Hutch *Pittosporum mannii* Hook.f. *Salvadora persica* L.	All species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Senna italica Mill. *Urtica dioica* L. *Warburgia ugandensis* Sprague	*Senna italica* Mill. *Urtica dioica* L. *Warburgia ugandensis* Sprague *Ajuga remota* Benth. *Amaranthus dubius* Mart. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Balanites glabra* Mildbr. & Schltr. *Cynodon aethiopicus* Clayton & Harlan *Melinis repens* (Willd.) Zizka *Musa acuminata* Colla *Panicum miliaceum* L. *Pennisetum glaucum* (L.) R.Br. *Secale cereale* L. *Senna occidentalis* (L.) Link *Sesamum indicum* L. *Sorghum bicolor* (L.) Moench T*riticum aestivum* L. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. *Triticum turgidum* L. *Zea mays* L.	
27	MAN37	7	*Albizia anthelmintica* Brongn *Dombeya rotundifolia* (Hochst.) Planch.	*Albizia anthelmintica* Brongn *Dombeya rotundifolia* (Hochst.) Planch.	Only 6 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Olea europaea L. *Osyris lanceolata* Hochst. & Steud. *Rhamnus prinoides* L'Hér. *Warburgia ugandensis* Sprague *Zanthoxylum usambarense* (Engl.) Kokwaro	*Olea europaea* L. *Osyris lanceolata* Hochst. & Steud. *Warburgia ugandensis* Sprague *Zanthoxylum usambarense* (Engl.) Kokwaro *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Cucurbitaceae sp.* *Solanum lycopersicum* L. *Solanum melongena* L. *Teclea simplicifolia* (Engl.) I. Verd. *Vachellia drepanolobium* (Harms ex Sjostedt) P.J.H. Hurter *Zea mays* L.	
28	MAN39	3	*Moringa oleifera* Lam. *Ocimum gratissimum* L. *Rosmarinus officinalis* L	*Moringa oleifera* Lam. *Ocimum gratissimum* L. *Rosmarinus officinalis* L *Dactyloctenium aegyptium* (L.) Willd. *Pennisetum glaucum (*L.) R.Br. *Secale cereale* L. *Triticum aestivum* L. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. *Triticum turgidum* L. *Zea mays* L.	All species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
29	MAN41	7	*Albizia anthelmintica* Brongn *Carissa edulis* Forssk. *Lantana camara* L. *Osyris lanceolata* Hochst. & Steud. *Rhamnus prinoides* L'Hér. *Rotheca myricoides* (Hochst.) Steane & Mabb. *Warburgia ugandensis* Sprague	*Albizia anthelmintic*a Brongn *Carissa edulis* Forssk. *Lantana camara* L. *Osyris lanceolata* Hochst. & Steud. *Rhamnus prinoides* L'Hér. *Rotheca myricoides* (Hochst.) Steane & Mabb. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Faurea saligna* Harv. *Rutaceae sp.* *Zanthoxylum sp.* *Zea mays* L.	All species on label were detected
30	MAN42	12	*Albizia anthelmintica* Brongn *Carissa edulis* Forssk. *Clerodendrum heterophyllum* (Poir.) R.Br. *Faurea saligna* Harv. *Lantana camara* L. *Olea europaea* L. *Osyris lanceolata* Hochst. & Steud. *Pappea capensis* Eckl. & Zeyh. *Rhamnus prinoides* L'Hér. *Rhamnus staddo* A.Rich.	*Albizia anthelmintica* Brongn *Carissa edulis* Forssk. *Clerodendrum heterophyllum* (Poir.) R.Br. *Faurea saligna* Harv. *Lantana camara* L. *Olea europaea* L. *Osyris lanceolata* Hochst. & Steud. *Pappea capensis* Eckl. & Zeyh. *Rhamnus prinoides* L'Hér. *Rhamnus staddo* A.Rich.	All species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Rotheca myricoides (Hochst.) Steane & Mabb. *Zanthoxylum chalybeum* Engl.	*Rotheca myricoides* (Hochst.) Steane & Mabb. *Zanthoxylum chalybeum* Engl. *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Aloe vera* (L.) Burm.f. *Rutaceae sp.* *Triticum aestivum* L. *Triticum turgidum* L. *Zanthoxylum sp.* *Zea mays L*.	
31	MAN43	8	*Albizia anthelmintica* Brongn *Croton dichogamus* Pax *Euclea divinorum* Hiern *Pappea capensis* Eckl. & Zeyh. *Pittosporum mannii* Hook.f. *Salvadora persica* L. *Senna italica* Mill. *Teclea simplicifolia* (Engl.) I. Verd.	*Albizia anthelmintica* Brongn *Croton dichogamus* Pax *Euclea divinorum* Hiern *Pappea capensis* Eckl. & Zeyh. *Pittosporum mannii* Hook.f. *Salvadora persica* L. *Senna italica* Mill. *Ajuga remota* Benth. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Ocimum gratissimum* L. *Rutaceae sp.* *Rosmarinus officinalis* L *Senna occidentalis* (L.) Link	Only7 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
				Zanthoxylum sp. *Zea mays* L.	
32	MAN45	4	*Azadirachta indica* A.Juss. *Moringa oleifera* Lam. *Prunus africana* (Hook.f.) Kalkman *Sesamum indicum* L.	*Azadirachta indica* A.Juss. *Moringa oleifera* Lam. *Sesamum indicum* L. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Gynandropsis gynandra* (L.) Briq. / Cleome gynandra L. *Ipomoea batatas* (L.) Lam. *Jatropha curcas* L. L. *Leucaena leucocephala* (Lam.) de Wit *Ocimum basilicum* L.	Only 3 species on label were detected
33	MAN46	4	*Hibiscus flavifolius* Ulbr.(Hibiscus Mentha (mint) *Rosmarinus officinalis* L (rosemary) *Urtica dioica* L. (stinging neetle)	*Urtica dioica* L. *Urtica massaica* Mildbr.	Only1 species on label were detected
34	MAN47	9	*Aloe secundiflora* Engl. *Carissa edulis* Forssk. *Croton megalocarpus* Hutch *Heuchera micrantha* Douglas ex Lindl.(Alum roots) alam roots *Myrsine africana* L. *Prunus africana* (Hook.f.) Kalkman	*Albizia anthelmintica* Brongn *Amaranthus dubius* Mart. *Balanites glabra* Mildbr. & Schltr. *Carica papaya* L. *Cynodon aethiopicus* Clayton & Harlan *Galinsoga parviflora* Cav.	No species on label detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Stellaria media (L.) Vill.(chicken weed) *Taraxacum officinale* (L.) Weber ex F.H.Wigg.(Dandelion) *Urtica dioica* L.	*Ipomoea wightii* *Micromeria imbricata* *Neoachmandra cunninghamii* *Ocimum gratissimum* L. Oryza sativa L. *Pennisetum glaucum* (L.) R.Br. *Persea americana* Mill. *Pittosporum mannii* Hook.f. *Secale cereale L.* *Senna occidentalis* (L.) Link *Triticum turgidum* L. *Zea mays* L. *Zehneria sp.*	
35	MAN48	3	*Strychnos henningsii* Gilg *Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f.	*Strychnos sp.* *Albizia anthelmintica* Brongn *Aloe vera (L.)* Burm.f. *Carica papaya L.*	Only 2 species on label were detected
36	MAN49	8	*Aloe secundiflora* Engl. *Albizia anthelmintica* Brongn *Myrsine africana* L. *Pennisetum glaucum* (L.) R.Br. *Urtica dioica* L.	*Aloe secundiflora* Engl. *Albizia anthelmintica* Brongn *Myrsine africana* L. *Pennisetum glaucum* (L.) R.Br. *Urtica dioica* L.	Only 7 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Acacia kirkii Oliv. *Warburgia ugandensis* Sprague *Zanthoxylum usambarense* (Engl.) Kokwaro	*Acacia kirkii* Oliv. *Warburgia ugandensis* Sprague *Ajuga remota* Benth. *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Aloe vera* (L.) Burm.f. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Carica papaya* L. *Cynodon dactylon* (L.) Pers. *Musa acuminata* Colla *Pittosporum mannii* Hook.f. *Saccharum officinarum* L. *Senna italica* Mill. *Senna occidentalis* (L.) Link *Solanum melongena* L. *Strychnos sp*. *Zea mays* L.	
37	MAN50	6	*Albizia anthelmintica* Brongn *Azadirachta indica* A.Juss. *Euclea divinorum* Hiern *Toddalia asiatica* (L.) Lam. *Trigonella foenum-graecum* L (Fenugreek)	*Albizia anthelmintica* Brongn *Azadirachta indica* A.Juss. *Euclea divinorum* Hiern *Trigonella foenum-graecum* L *Vernonia brachycalyx* O.Hoffm.	Only 4 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Vernonia brachycalyx O.Hoffm.	*Aloe vera* (L.) Burm.f. *Annonaceae sp.* *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Carissa edulis* Forssk. *Coriandrum sativum* L. *Cuminum cyminum* L. *Hordeum vulgare* L. *Oryza barthii* *Oryza glaberrima* *Oryza meridionalis* *Pittosporum mannii* Hook.f. *Senna italica* Mill. *Senna occidentalis* (L.) Link *Triticum aestivum* L. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. *Triticum turgidum* L.	
38	MAN51	6	*Acacia mellifera* (M.Vahl) Benth. *Catharanthus roseus* (L.) G.Don (Periwinkal leaves) *Cymbopogon citratus* (DC.) Stapf (Lemon grass) *Heuchera micrantha* Douglas ex Lindl.(Alum roots) alam roots	*Cymbopogon citratus* (DC.) Stapf *Rumex crispus* L. *Acacia kirkii* Oliv. *Annonaceae sp.*	Only 2 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Hypericum perforatum L. (John wort)	*Dombeya rotundifolia* (Hochst.) Planch.	
			Rumex crispus L. (Yellow dock)	*Ocimum gratissimum* L. *Oryza sativa L.* *Osyris lanceolata* Hochst. & Steud. *Secale cereale* L. *Urtica dioica* L. *Zea mays* L.	
39	MAN52	4	*Albizia anthelmintica* Brongn *Fuerstia africana* T.C.E.Fr. *Salvadora persica* L. *Warburgia ugandensis* Sprague	*Albizia anthelmintica* Brongn *Salvadora persica* L. *Warburgia ugandensis* Sprague *Balanites glabra* Mildbr. & Schltr. *Ipomoea batatas* (L.) Lam. *Urtica dioica* L.	Only 3 species on label were detected
40	MAN54	8	*Albizia anthelmintica* Brongn, *Annona muricata* L. (Graviola fruit powder) *Cucurbita pepo* L. (pumkin seeds) *Linum usitatissimum* L. (linseeds) *Phyllanthus emblica* L. (Indian goosebery) *Pinus sp* (pine bark) *Prunus africana* (Hook.f.) Kalkman *Rumex crispus* L. (yellow dock)	*Albizia anthelmintica* Brongn *Annonaceae sp.* *Rumex crispus* L. *Anacardiaceae sp.* *Balanites glabra* Mildbr. & Schltr. *Ocimum gratissimum* L. *Secale cereale* L. *Triticum aestivum* L.	Only 2 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
				Triticum dicoccoides (Körn. ex Asch. & Graebn.) Schweinf.	
				Triticum turgidum L. *Zea mays* L.	
41	MAN55	3	*Rhamnus prinoides* L'Hér. *Urtica massaica* Mildbr. *Amaranthus dubius* Mart.	*Osyris lanceolata* Hochst. & Steud.	No species on label detected
42	MAN56	6	*Clausena anisata* (Willd.) Hook.f. ex Benth. *Osyris lanceolata* Hochst. & Steud. *Salvadora persica* L. *Schinus molle* L. *Urtica dioica L.* *Warburgia ugandensis* Sprague	*Clausena anisata* (Willd.) Hook.f. ex Benth. *Osyris lanceolata* Hochst. & Steud. *Salvadora persica* L. *Urtica dioica* L. *Warburgia ugandensis* Sprague *Ajuga remota* Benth. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Nicotiana tabacum* L. *Ocimum gratissimum* L. *Oryza sativa* L. *Rosmarinus officinalis* L *Zea mays* L.	Only 5 species on label were detected
43	MAN57	5	*Ajuga remota* Benth. *Albizia anthelmintica* Brongn *Faurea saligna* Harv.	*Ajuga remota* Benth. *Faurea saligna* Harv. *Rhamnus staddo* A.Rich.	Only 4 species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Rhamnus staddo A.Rich.	*Rotheca myricoides* (Hochst.) Steane & Mabb.	
			Rotheca myricoides (Hochst.) Steane & Mabb.	*Amaranthus dubius* Mart. *Dodonaea viscosa* (L.) Jacq. *Zea mays* L.	
44	MAN58	4	*Aloe vera* (L.) Burm.f. *Moringa oleifera* Lam. *Warburgia ugandensis* Sprague *Withania somnifera* (L.) Dunal	*Aloe vera* (L.) Burm.f. *Moringa oleifera* Lam. *Warburgia ugandensis* Sprague *Withania somnifera* (L.) Dunal *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Avena sativa* L. *Azadirachta indica* A.Juss. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Balanites glabra* Mildbr. & Schltr. *Galinsoga parviflora* Cav. *Osyris lanceolata* Hochst. & Steud. *Rosmarinus officinalis* L *Triticum aestivum* L. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. *Triticum turgidum* L. *Urtica dioica* L.	All species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
				Zea mays L. *Zehneria sp.*	
45	MAN59	4	*Aloe vera* (L.) Burm.f. *Moringa oleifera* Lam. *Warburgia ugandensis* Sprague *Withania somnifera* (L.) Dunal	*Aloe vera* (L.) Burm.f. *Moringa oleifera* Lam. *Warburgia ugandensis* Sprague *Withania somnifera (*L.) Dunal *Albizia anthelmintica* Brongn *Aloe tormentorii* (Marais) L.E.Newton & G.D.Rowley *Amaranthus dubius* Mart. *Azadirachta indica* A.Juss. *Baccharoides calvoana* (Hook.f.) "Isawumi, El-Ghazaly & B.Nord." *Croton dichogamus* Pax *Gynandropsis gynandra* (L.) Briq. / *Cleome gynandra* L. *Oryza sativa* L. *Senna occidentalis* (L.) Link *Sesamum indicum* L. *Triticum aestivum* L. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. *Triticum turgidum* L. *Urtica dioica* L.	All species on label were detected
46	MAN60	4	*Ajuga remota* Benth.	*Ajuga remota* Benth.	All species on label were detected

S. No.	Product code	Listed species	Species name indicated on label	Detected species using metabarcoding	Detected species as listed
			Albizia anthelmintica Brongn *Pittosporum mannii* Hook.f.	*Albizia anthelmintica* Brongn *Pittosporum mannii* Hook.f.	
			Salvadora persica L.	*Salvadora persica* L. *Secale cereale* L. *Sorghum bicolor* (L.) Moench *Zea mays* L.	
47	MAN61	8	*Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Amaranthus dubius* Mart. *Faurea saligna* Harv. *Pittosporum mannii* Hook.f. *Rotheca myricoides* (Hochst.) Steane & Mabb. *Warburgia ugandensis* Sprague *Ximenia americana* L.	*Albizia anthelmintica* Brongn *Aloe vera* (L.) Burm.f. *Amaranthus dubius* Mart. *Faurea saligna* Harv. *Pittosporum mannii* Hook.f. *Rotheca myricoides* (Hochst.) Steane & Mabb. *Warburgia ugandensis* Sprague *Anacardiaceae sp.* *Capsicum annuum* L. *Croton megalocarpus* Hutch *Oryza sativa L.* *Pennisetum glaucum* (L.) R.Br. *Salvadora persica* L. *Senna italica* Mill. *Sorghum bicolor* (L.) Moench *Urtica dioica* L. Zanthoxylum sp. *Zea mays* L.	Only 7 species on label were detected

Appendix VI: Regression analysis of reads and MOTUs produced

SUMMARY OUTPUT

Regression Statistics

Multiple R	0.417
R Square	0.174
Adjusted R Square	0.156
Standard Error	117772.774
Observations	47

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.32E+11	1.32E+11	9.495851	0.00350854
Residual	45	6.24E+11	13870426208		
Total	46	7.56E+11			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	33409.261	33010.266	1.012	0.317	-33076.827	99895.348	-33076.827	99895.348
Average MOTU	1530.755	496.751	3.082	0.004	530.247	2531.263	530.247	2531.263

Appendix VII: Regression analysis of detected versus adulteration species

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.866526
R Square	0.750868
Adjusted R Square	0.745332
Standard Error	2.801483
Observations	47

ANOVA

	df	***SS***	***MS***	***F***	***Significance F***
Regression	1	1064.443	1064.443	135.627	3.56E-15
Residual	45	353.1739	7.848309		
Total	46	1417.617			

	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***	***Lower 95.0%***	***Upper 95.0%***
Intercept	2.960981	0.761682	3.887423	0.00033	1.426874	4.495087	1.426874	4.495087
X Variable 1	1.221645	0.104899	11.6459	3.56E-15	1.010368	1.432923	1.010368	1.432923

Appendix VIII: Research Authorisation from Graduate School

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: I84/31033/15

Date: 10th November, 2016

The Director General,
National Commission for Science, Technology & Innovation,
P.O. Box 30623-00100,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MS.MWAURA ANN REG. NO. I84/31033/15

I write to introduce Ms. Mwaura who is a Postgraduate Student of this University. She is registered for a Ph.D. degree programme in the **Department of Plant Sciences in the School of Pure & Applied Sciences.**

Ms. Mwaura intends to conduct research for Ph.D. thesis entitled, **"Identification of Commonly used Medicinal Plant Species in Kenya using DNA Barcoding"**

Any assistance given will be highly appreciated.

KENYATTA UNIVERSITY
OFFICE OF DEAN
10 NOV 2016
GRADUATE SCHOOL
P.O. Box 43844-00100

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

RM/cao

Appendix IX: Approval of Research Proposal

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel: 81091 Ext. 57530

KENYATTA UNIVERSITY 11 NOV 2016 P.O. Box 43844-00100

Internal Memo

FROM: Dean, Graduate School

DATE: 10th November, 2016

TO: Ms. Mwaura Ann
C/o Department of Plant Sciences
KENYATTA UNIVERSITY

REF: I84/31033/15

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the Graduate School Board at its meeting 10th November, 2016 approved your Ph.D. Research Proposal **entitled "Identification of Commonly used Medicinal Plant Species in Kenya using DNA Barcoding"**

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking Forms per semester. The form has been developed to replace the progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

By copy of this letter, the Registrar (Academic) is hereby requested to grant you substantive registration for your Ph.D. studies.

KENYATTA UNIVERSITY OFFICE OF DEAN 10 NOV 2016 GRADUATE SCHOOL P.O. Box 43844-00100

Thank you.

REUBEN MURIUKI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Plant Sciences
Registrar (Academic) Att; Mr. Likam
Supervisors:

1. Dr. Joseph Kamau
   C/o Plant Sciences
   KENYATTA UNIVERSITY
2. Dr. Barbara Gravendel
   Naturalis Biodiversity Center, Leiden, the Netherlands
   C/o Department of Biochemistry & Biotechnology
   KENYATTA UNIVERSITY
3. Dr. Omwoyo Ombori
   C/o Department of Plant Sciences
   KENYATTA UNIVERSITY

Appendix X: Research Permit from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 406505

Date of Issue: 16/April/2021

RESEARCH LICENSE

This is to Certify that Ms.. Ann Njeri Mwaura of Kenyatta University, has been licensed to conduct research in Kajiado, Nairobi, Narok on the topic: Identification of Commonly Used Medicinal Plant Species in Kenya Using DNA Barcoding for the period ending : 16/April/2022.

License No: NACOSTI/P/21/9844

406505

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013

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Appendix XI: Publication

EISSN: 2707-0425

East African Journal of Science, Technology and Innovation, Vol. 1 (3): 2020.

An ethnobotanical study of medicinal plants commonly traded in Kajiado, Narok and Nairobi counties, Kenya

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Abstract

Over eighty percent of the world population depend on traditional medicine for their basic health care needs. A study was carried out in three counties in Kenya (Kajiado, Narok and Nairobi) to document the common plant species traded as medicinal or herbal remedies. Structured interviews and questionnaire were administered to herbalists with prior informed consent, who were willing to disclose information on the source, plant type and parts of the herbal medicine they were selling and ailments treated. The folk or common names were recorded and later translated to scientific names using para-taxonomists and previous published data. Majority of the herbalists interviewed were between the ages of 40-59 years and comprised of mostly women (54%). The investigations revealed that eighty-six (86) plant species were traded as medicinal plants out of which 51% were commonly traded across the three counties. The study further revealed that the most traded plant parts were stem, bark and roots which could pose a threat to conservation of the species due to complete or partial destruction of the trees during harvesting. Aloe species, *Prunus africana* and *Osyris lanceolata* were highly traded an indication of their preference by local inhabitants to treat particular ailments. The generated list of medicinal plants species will form baseline data that could be used to generate a comprehensive list of all plant species traded as herbal medicine in Kenya. The commonly traded plants can also be included in pharmacological studies which may lead to development of new and potential drugs.

Keywords: *Kenya; herbalist; Medicinal plants; use value; ailments*

Cite as: *Mwaura et al, 2020* ***An ethnobotanical study of medicinal plants commonly traded in Kajiado, Narok and Nairobi counties, Kenya*** *East African Journal of Science, Technology and Innovation 1(3)*

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Introduction

The extensive use of traditional medicine in Africa, composed mainly of medicinal plants, has been argued to be linked to cultural and economic reasons. The World Health Organization (WHO) documented that between 65 % and 80 % of the population in developing countries use plants as medicinal remedies since modern medicine is not readily available (Karimi *et al.*, 2015; Nisar *et al.*, 2018). Globally, there is a rising trend to shift resources from allopathic to traditional healthcare systems due to the increasing cost of modern drugs coupled with the decline in the purchasing power of the people. This has been caused by the declining economic opportunities especially in rural areas that has made it mandatory for governments to intensify efforts towards documentation and research on medicinal plants. The global market for medicinal and aromatic plants was US$ 62 billion in 2002 and estimates suggest that it will reach US$ 5 trillion by 2050. Traditional complementary and alternative medicine like herbal medicines forms an integral part of primary health care in Kenya (Okumu *et al.*, 2017). Over 80 % of the population use herbal remedies as a fundamental component of the healthcare system (Fowler, 2006; WHO, 2011; Randriamiharisoa *et al.*, 2015). By the year 2011, Kenya had forty thousand traditional healers including herbalists, bone setters, faith healers