ETHNOBOTANICAL AND TAXONOMIC STUDY OF *CATHA EDULIS* PLANT GROWN IN EMBU AND MERU COUNTIES, KENYA.

BY

KIUNGA JOSPHAT KINYUA

BSc. (Hons)

UNIVERSITY OF NAIROBI.

I56/69745/2013

A THESIS SUBMITTED TO THE SCHOOL OF BIOLOGICAL SCIENCES IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT TAXONOMY AND ECONOMIC BOTANY OF THE UNIVERSITY OF NAIROBI

DECLARATION

I **<u>KIUNGA JOSPHAT KINYUA </u>(I56/69745/2013)** hereby declare that this thesis is my original work and has not been submitted for a degree in any other university to the best of my knowledge.

Signed……... …………………… Date……………………..

This thesis has been submitted for examination with our approval as university supervisors:

1. Dr. Catherine W.Lukhoba (Ph.D.)
School of Biological Sciences
University of Nairobi

Signed…………………………… Date…………………………

2. Prof. Saifuddin F.Dossaji (Ph.D.)
School of Biological Sciences
University of Nairobi

Signed………………………… Date……………………….

3. Prof. Abiy Yenesew (Ph.D.)
Department of Chemistry
University of Nairobi

Signed…………………………. Date………………………

DEDICATION

This work is dedicated to my grandfather Mr. James Kiunga, and Ms. Lucy M. Mwenda who have played the role of a mother and a mentor to me. Both of them have been a great source of inspiration in my life.

ACKNOWLEDGEMENTS

First, I would like to thank the University of Nairobi for the scholarship which allowed me to pursue my M.Sc.studies.I would also like to wholeheartedly thank my supervisors for their generous support throughout the study. In particular, I would like to sincerely acknowledge the support given to me by Dr. Catherine Lukhoba who constantly provided guidance in this work and ensured that all the materials needed for taxonomic work were available. Her contribution towards the success of this work was immense and I will truly remain indebted to her for this and also for her moral support.

Many thanks go to Prof. Abiy Yenesew for his guidance and also for allowing me to use his laboratory for phytochemical analysis as well as providing all the reagents, chemicals and apparatus that were required for TLC analyses. I would not have made it were it not for his generous noble gesture. I would also like to thank Prof. Saifuddin Dossaji for his patience, guidance and positive criticism of the work.

I want to thank the people of Meru and Embu counties with whom and for whom this project was carried out. Their hospitality and keen interest in the proceedings was an inspiration during field work. Many have contributed to the information compiled in this document. I would also like to thank the East African Herbarium for allowing me to access herbarium specimens and conduct part of my studies in the herbarium. My life at the University of Nairobi would not have been complete, were it not for the social buffer created by colleague graduate Students. Last but not the least, I thank all my close relatives and friends who have supported, encouraged and inspired me throughout this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

DCM	Dichloromethane
EtOAc	Ethyl acetate
FTEA	Flora of Tropical East Africa

GPS	Geographical Position System
MeOH	Methanol
NACOSTI	National Commission for Science, Technology and Innovation
R_f	Retention Factor
TLC	Thin Layer Chromatography
O.T.U	Operation Taxonomic Unit
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
SPSS	Statistical Package for Social Sciences

ABSTRACT

Catha edulis (Vahl) Forssk.ex Endl. (Celastraceae) is a commercially important plant whose young leaves and stems are chewed as a psychostimulant in East Africa and some Arabian Countries such as Yemen and Afghanistan. However, the information on taxonomy and ethno-medicinal uses of *C.edulis* other than its known use as a psychostimulant drug remains poorly known. The present study was designed to document medicinal uses in addition to establishing specific criteria of discriminating various traditional varieties by use of morphological and chemical characters. This ethnobotanical survey was conducted between the month of September and December (2014) in Meru and Embu counties. The ethnomedicinal information was gathered through face to face interviews of forty-two key informants (32 males and 10 females) aged between 45 and 84years while the morphological data was collected through field observations and laboratory evaluation of the collected plant samples. Thirty three characters/character states (both vegetative and floral) selected using fifteen traditional varieties of *C.edulis* were subjected to numerical taxonomic analysis. The methanolic extracts of fresh leaves and young stems of *C.edulis* were subjected to acid-base extraction and the basic organic extracts analyzed by TLC for the presence of khatamines. Thirteen diseases/ill-health conditions were reported to have been cured through use of *C.edulis* material.Of these, 62% were reported in Meru County while 23% were reported in both Meru and Embu counties. The remaining 15% of the total ethnomedicinal uses of *C.edulis* were reported in Embu County. An elaborate nomenclatural system and classification of *C.edulis* by Ameru and Aembu communities of Kenya exists based on characters such as leaf/stem colour, leaf shapes as well potency levels. Five traditional varieties from Meru county namely: K*iraa gikiiru, kigwe, muchuri ,kithaara, kilantune*, and six traditional varieties from Embu county namely: *muguka, kibwe, muti-mutiri, mugumo, mugwathingi, gitu* were identified based on ethnomedicinal studies and their traditional classification given. The UPGMA grouped the fifteen traditional varieties of *C.edulis* studied into two main clusters. The clusters segregated geographically with the first cluster comprising of all traditional varieties collected in Embu County namely:*Kibwe, mugwathingi, gitu, muti-mutiri, mugumo, muguka A, muguka B* while the second cluster comprised of all the traditional varieties of *C.edulis* collected in Meru County namely: *Kiraa gikiiru A, Kiraa gikiiru B, Kiraa gikiiru* C,*kigwe, kithaara, kilantune, Muchuri*. Results of chemotaxonomic analysis based on TLC method appeared to corroborate the morphological data above as three compounds were detected in fresh young stem extracts of all the traditional varieties from Meru County while two compounds were detected in fresh young stems of all the traditional varieties collected in Embu County. Profiles of the fresh young leaf extracts of all the traditional varieties from both counties revealed the presence of two compounds upon TLC analyses. The fifteen traditional varieties provided by farmers/consumers, two major groupings based on numerical taxonomic methods and chemotaxonomy clearly indicate that the species *C.edulis* is heterogeneous and hence the possible existence of infraspecific taxa within the species. A comprehensive morphological study covering all collections from other parts of the country such as Western Kenya and North Eastern parts of Kenya and including wild samples should be carried out to make a more thorough classification.

Key words: Khat, Psychostimulant, Numerical taxonomy, Chemotaxonomy.

CHAPTER ONE

INTRODUCTION

1.1 Background

Catha edulis (Vahl) Forssk.exEndl (Celastraceae) is a species of evergreen trees or shrubs (Curto *et al*., 2013) cultivated widely in Eastern Africa and some parts of Arabian Peninsula (Saif-Ali *et al*., 2003).In Kenya, the plant is extensively cultivated in Meru and Embu counties. It is a popular psychostimulant plant which has elicited heated debate in recent years, both locally and internationally, dueto its socio-economic impact. The principal psychoactive constituents of *C.edulis* are cathinone and cathine which have sympathomimetic actions (Kalix and Braenden, 1985).These compounds are believed to be concentrated mainly in young leaves of *C.edulis* (WHO, 2006; Szendrei, 1980).The trade of *C.edulis* material occurs openly in Kenya, Djibouti, Ethiopia, Somalia, Uganda, and Yemen but it is illegal in Eritrea, Kuwait, Saudi Arabia, Sudan, Tanzania, and Zambia (Gerstle, 2007). Somalia has the highest percentage of khat consumers in the world with most of the khat consumed in this country being imported from Kenya (Perlez, 1992).The most common adverse effects of chewing *C.edulis* material include insomnia, anorexia, hyperthermia, mydriasis, endocrinological disturbances and acute autonomic responses, such as elevated blood pressure and tachycardia (Hassan *et al*., 2000).

There are a few reports on ethnomedicinal uses of *C.edulis* although the psychostimulating effects and behavioral changes associated with chewing of the plant material are well known. Furthermore, the limited studies that have been carried out on ethnomedicinal uses of this plant have often yielded conflicting information. For example, Kokwaro (2009) reported the use of khat in the treatment of stomach upset. On the contrary, Nigussie *et al*. (2013) reported a close association between khat chewing and gastrointestinal disorders.

In recent years, ethnobotanical studies have facilitated the exploration of morphological, chemical and genetic characters of plants for human benefits. Ethnobotany, which is the study of relationships between plants and people (Rahman, 2013), has not been done extensively on

C.edulis of Kenyan origin primarily cultivated in Meru and Embu Counties to account for the possible diversity of *C.edulis* germplasm and ethnomedicinal value based on traditional knowledge. A few reports on traditional diversity of *C.edulis* of Kenyan origin exist in literature. For instance, Carrier (2007) mentions four traditional varieties grown in Embu and Meru counties namely: *miraamiiru, kilantune*, *muguka* and *kiandasi*, that are distinguishable by shades of colour, habit and stimulating potency levels.

Taxonomically, there is block categorization of the species *C.edulis* although recent molecular and chemical studies of *C.edulis* from different geographical origins and even traditional knowledge all indicate possible infraspecific variations within the species. The morphological characters of *C.edulis* at specific level are well known (Kalix 1984; Kalix and Braenden, 1985; Robson*et al*., 1994). Nevertheless, even with existence of wide range of morphological characteristics of *C.edulis* such as leaf characters and quantitative characters of the plant, they are not sufficiently discontinuous to allow discrimination of *C.edulis* at infraspecific level.This calls for the use of more sensitive techniques such as numerical techniques to delimit the species at infraspecific level(Deshmukh, 2011).Given that no attempt has been made so far to determine the taxonomic status of *C.edulis* by use of numerical methods, there is a need to investigate this phenomenon by use of as many character states as possible with the sole aim of delimiting possible infraspecific taxa of *C.edulis* of Kenyan origin.

Plants produce an array of phytochemicals some of which are essential for their survival (primary metabolites) while others do not have primary functions but may play a role in defence mechanisms (secondary metabolites).These secondary metabolites differ in type and concentration in various plant taxa and are useful in taxonomy.Only a few chemical studies have been carried out to determine the various chemotypes of *C.edulis* based on the content of khatamines in *C.edulis* from different geographical origin. Geisshusler and Brenneisen(1987) investigated the content of khatamines in *C.edulis* involving twenty two samples from Kenya,Ethiopia,Northern Yemen and Madagascar. They found out that *C.edulis* differed in alkaloid content depending on the geographical origin of the khat samples. The study was, however, inconclusive as it did not take into account the distribution of the khatamines across various consumable parts of the species *C.edulis* such as leaves and young stems. Attention was paid on leaves only. The study was also not localized in one country and this would have resulted in environmental bias. In Kenya, the consumed parts of *C.edulis* are leaves in some cases and young twigs in other cases depending on the geographical origin of khat mainly from Embu and Meru counties.

Questions that may be addressed through chemical study of leaves and stems of *C.edulis* are why some traditional varieties are claimed to be more potent than others and why some people prefer leaves while others prefer young stems. Essentially, chemical study would help elucidate the old-age habit of preference of leaves in some cases and young stems in other cases as well as support numerical taxonomic analysis of different traditional varieties of *C.edulis* from Meru and Embu counties of Kenya.

Given the widespread availability and use of *C.edulis*, a rapid method to identify khat is necessary for law enforcement agencies. In addition, alternative uses of khat such as ethnomedicinal uses need to be investigated for a sound evaluation of the full economic potential of the plant. The present study was therefore designed to establish taxonomic position of *C.edulis* as well as determine its ethnomedicinal uses in Meru and Embu counties of Central Kenya.

1.2 Problem statement

During the last two decades, important progress has been made in understanding the pharmacological and social effects of khat in Kenya. However, little attention has been paid on the medicinal aspects, taxonomy and also the chemistry of different traditional varieties of *C.edulis*. Previous medicinal studies are scanty and often contradictory. For instance, some authors have reported *C.edulis* to be a source of remedy for gastrointestinal disorders (Kokwaro, 2009) while in other cases, consumption of *C.edulis* material has been closely associated with gastrointestinal disorders (Nigussie *et al*., 2013). These and many more allude to inadequate and often conflicting information on the ethno-medicinal uses of *C.edulis.*

Different traditional varieties of *C.edulis* have different pharmacological action (Al-Thobhani *et al*., 2008). However, there is no study that has been dedicated so far to re-examine the taxonomic status of *C.edulis* with regard to infraspecific variations that could be in existence within the species. The most current morphological classification of *C. edulis* (Robson *et al.*, 1994) did not consider infraspecific taxa of *C.edulis* although there is possible existence of blatant differences based on recent genetic and chemical information sources. In Kenya, little is known about the taxonomy and the distribution of traditional varieties mentioned by Carrier (2007). Also, the number of possible varieties that could exist in Meru and Embu counties, where *C.edulis* is extensively cultivated, is not clear and therefore there is a need for thorough investigation of ethnotaxonomy of *C.edulis* by the Meru and Embu people.

The use of numerical techniques was envisaged to provide a greater discrimination power along the spectrum of taxonomic differences as it is a rather more sensitive technique in delimiting taxa (Pinheiro and de Barros, 2007).The previous chemical studies were also incoherent as they never took into account variations that could exist among different traditional varieties of *C.edulis* as well as different parts of the plant that are consumed, that is, young leaves and stems.The present study was therefore designed to document ethno medicinal uses, numerical taxonomy as well as chemotaxonomy of *C.edulis.*

1.3 Objectives

1.3.1 General objective

The general objective of this work was to collate the ethnomedicinal uses and determine the taxonomic position of *Catha edulis* found in Meru and Embu counties of Kenya

1.3.2 Specific objectives

1. To determine ethnomedicinal uses and traditional classification of *C.edulis* species in Meru and Embu counties of Kenya.
2. To carry out morphological study of traditional varieties of *C.edulis* species from different localities and identify possible varieties found in Meru and Embu Counties of Kenya.
3. To conduct a chemotaxonomic analysis of *C.edulis* from Meru and Embu counties.

1.4 Justification

In Kenya, *Catha edulis* is one of the most economically important plants along with tea and coffee. The government of Kenya generates large amount of revenue through the sale of *C.edulis* material both locally and internationally. It is also the main economic activity in Meru County where it is extensively cultivated and also some parts of Embu County.Many Kenyans are employed in *C.edulis* industry.The export and local sale of khat material generates approximately $150 million annually in Kenya(Nyongesa and Onyango(2010).

The species *C.edulis* is implicated for its medicinal uses. So far, very limited studies have been carried out on the medicinal value of *C.edulis* of Kenyan origin. In addition, the limited studies that have been carried out on medicinal aspects of *C.edulis* have yielded conflicting information. However, besides being a major source of income in the communities where it is extensively cultivated and the government generating revenue through trade of this crop, *C.edulis* species has been reported to have long term and short term detrimental health effects on the users. These include effects on central nervous system, reproductive system, respiratory system among others. It is not known whether the traditional varieties of the species *C.edulis* have the same or different physiological effects.

There is practically no numerical taxonomy or chemotaxonomic work that has been carried out in Kenya to explain the possible infraspecific variations that could exist within the species.*C. edulis* is a good model for ethnomedicinal and taxonomic study due to its socio-economic impacts on Aembu and Ameru communities of Kenya where it is extensively cultivated.

1.5 Research hypothesis

The traditional knowledge of ethnobotanical uses and properties of traditional varieties of *C.edulis* is uniform and that morphological characters, quality and quantity of khatamines of *C.edulis* are distributed uniformly in different traditional varieties found in Embu and Meru counties of Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical use of *Catha edulis*

Catha edulis (Vahl) Forssk.exEndl. (Celastraceae) is a plant of antiquity associated with psychological, health and socio-economic issues on humans (Karunamoorthi *et al*., 2010). The earliest references to this plant species dates back to the 15thcentury when the "Abyssinian tea" (Ethiopian tea) was noted as popular in Abyssinia and Aden (Margetts, 1967). This plant is known by a wide range of common names such as *miraa*, *kat*, *qat, chat*, *caftan* and bushman's tea (South Africa), Arabian tea, Abyssinian tea, African tea, flower of paradise, and so on. Greenway (1947) has provided a lengthy list of common names popular in most countries where this plant species occurs.

According to Karunamoorthi *et al*. (2010), *C.edulis* chewing is one of the most rampant longstanding age-old customs in Eastern and Southern Africa, and in parts of Arabian Peninsula such as Yemen The consumption of *C.edulis* material has crossed social, ethnic and religious barriers in the modern era, and it is futile to pinpoint a particular group of people and declare them *C.edulis* consumers (Goldsmith, 1988). Ancient Egyptians considered this plant to be holy,on top of being a cherished stimulant (Balint, 2012).*C.edulis* material has been designated as medically important plant since ancient times. Many authors believe that the earliest record of the medical use of *C.edulis* material is enshrined in the New Testament (Balint, 2012).

Alexander the Great is believed to have used *C.edulis* to treat his soldiers for an unknown epidemic. *C.edulis* is also mentioned in an Arabic medical book. Najeeb Al-Deen Al-Samargandi who is its author described *C.edulis* as a medical solution for depression because when consumed, it led to happiness and excitement (Krikorian, 1984). The historian Ibn Fadl Allah Al-Amri mentioned use of *C.edulis* during the war between Sabr-Al-Deen (the king of Ifat) and the King of Ethiopia (Amdetsion). After winning the war, King Sabr-Al-Deen vowed to bring down the residence of the Ethiopian king (Marade) and replace it with *C.edulis* plantation (Al-Motarreb *etal*., 2002).

2.2 Origin and Geographical Distribution of *Catha edulis*

According to National Commission for Science, Technology and Innovation (NACOSTI, 2006),*C.edulis* may have been growing in different regions of Eastern, Central and Southern Africa simultaneously. This presumption is highly supported by a wide range of vernacular names ascribed to this plant in different parts of Africa where it grows, which attests to a deeper understanding of the plant by local people in these regions. Another theory asserts that this plant species may claim its origin from Harar, Ethiopia and then later spread to other parts of the world (Krikorian, 1984; Getahun and Krikorian, 1973).

The Nyambene region in Meru county offer Kenyan *C.edulis* its ancestral home, according to Carrier (2007).Legend has it that the psychostimulating use of the *C.edulis* material was first discovered by a herder who noticed its effects on his goats and who experienced wakefulness and added strength after trying it and took some home for cultivation. Ethiopians know him by the name Awzulkernayien and even recite his name in their prayers while chewing *C.edulis* material (Krikorian, 1984).

Outside Africa, *C.edulis* is cultivated widely in Arabian Peninsula, Yemen, Afghanistan, India and Sri Lanka for consumption. It is grown in U.S.A, U.K. and France for experimental purposes (Lemessa, 2001).In Africa, the plant is widely distributed growing in habitats ranging from evergreen, submontane to deciduous woodlands (Nyongesa and Onyango, 2010). It is an indigenous plant in most African countries such as Kenya, Ethiopia, Eritrea, Somalia, Tanzania, Uganda, Burundi, Rwanda, Democratic Republic of Congo, Zambia, Zimbabwe, and South Africa. The plant occurs in altitudes ranging between 1680-2590 metres above the sea level (Getahun and Krikorian, 1973). In Kenya, *C.edulis* occurs naturally in Chyulu hills, Kericho, Slopes of Mt Kenya and Mt Elgon. It is extensively cultivated in Meru and Embu Counties (Carrier, 2007)

2.3 Taxonomy of *Catha edulis*

Catha edulis (Vahl) Forssk.ex Endl. belongs to the family Celastraceae R.Br. commonly known as the Bittersweet family. This is a family of woody trees, shrubs and lianas with a Gondwanaland distribution. The Celastraceae are morphologically poorly defined, but a natural family (Simmons and Hedin, 1999). Forsskal (1775) was the first to delineate the genus *Catha*. *Catha edulis* was published (posthumously) but not validated by Forrskal. Vahl (1790) took up the epithet and validly published the name *Celastrus edulis* citing *Catha edulis* Forssk in synonymy. However, the internationally accepted botanical name, *Catha edulis*, was first validly published by Endlicher (1841) hence the combination, *Catha edulis* (Vahl) Forssk.ex Endl., with the generic name '*Catha'* standing for Latinized version of the Yemeni word 'qat'for this plant and specific name '*edulis'* meaning edible(Krikorian,1985) because the plant was first seen being chewed rather than growing (Krikorian,1984).

Description of *Catha edulis*

Based on the latest revision of the family Celastraceae for the FTEA the following description is given for the species *C.edulis* by Robson *et al*. (1994):

"Tree 2-15(-25)m high; branches pale grey-green, flattened when young, becoming vinous red, terete; lamina dark-green or greyish green, glossy above, paler beneath, oblong/elliptic/obovate, leaf length(3.7-)5.5-11,width (0.8-)1.5-4.5(-6cm), acute/acuminate/obtuse, margin markedly glandular,crenulate,denticulate,cuneate/attenuate,coriaceous/subcoriaceous,reticulatevenation(more pronounced beneath than above, petiole 3-10mm long; inflorescence usually cymes,1-2-3cm long, dense, peduncle 6-12mmlong, pedicels very short or up to 2mm long, flowers many in each cyme,2-3mm in diameter, sepals broadly ovate to semicircular/rounded,0.5-0.7mm,margin ciliolate-fimbriate, petals elliptic-oblong,1-1.5mm long, sessile, margin ciliolate, paler in colour, stamens shorter than petals with filaments 0.7-1mm long; disc 1-1.5mm in diameter, ovary broadly ovoid, styles less than 0.5mm long and slender, stigma small, capsule red in colour, narrowly oblong 3-gonous,6-10mm,pendulous;seeds dark-brown, rugose-papillose with a narrow wing."No infraspecific taxa were considered for the species *Catha edulis* under this current classification.

2.4 Varieties of *Catha edulis* based on Traditional Knowledge.

Every community has its own way of perceiving discontinuities in nature based on obvious characters such as colour or medicinal value of plants. This subjective form of classification applies to plants of commercial value such as *C.edulis* for the purpose of grading them based on their qualities. For example, the Oromo people in Hararghe, Ethiopia, distinguish three varieties of *C.edulis* classified physically based on the colour of young shoots of the plant. These include:*dimaa* that has a red tinge, believed to withstand harsh conditions, can do well even with poor management, extensive harvest and gives reasonable harvest even when subjected to minimum care unlike other varieties; *Dalota* that is white/light in colour with narrow leaves and more branched,and Hamarcot that is in between *Dimaa* and *Dalota* in colour with broad leaves and less branched compared to *Dalota*, superior in quality and higher market value (Lemessa, 2001).

In Kenya, Carrier (2007) mentioned four traditional varietiesof *C.edulis* species distinguishable by shades of leaf color and potency levels found in Meru and Embu Counties which include *kilantune* (has a purple appearance cabbage-like taste), *miraamiiru*(balanced in taste and effects), *kiandasi* (very potent and chewers prone to a sensation of fleas crawling over their bodies) and *muguka* (mostpotent of all). The scientific investigation of these traditional varieties has not been carried out to determine their taxonomic position with regard to the species *C.edulis.*

2.5 Ethnobotany and its Significance

Ethnobotany is the study of classification, use and management of plants by people (Gary, 2004).Rahman (2013) defines ethnobotany as the study of the relationships between plants and people (From Ethno-study of people and -botany-study of plants).The focus of ethnobotany which is considered as a branch of ethnobiology (Rahman,2013) is on how plants have been used or continues to be used, managed and perceived in human societies, and includes plants that are potentially used as food, medicine, divination, cosmetics, dyes, textiles, for building, tools, currency, clothing, rituals and social life (Rahman, 2009).

The exploitation of plants by man for these benefits necessitated a folk knowledge of botanical classification and nomenclature, a branch of study known as ethnosystematics (Kokwaro, 1993). Ethnosystematics knowledge is highly developed in Kenya because of the rich and diverse flora (*ca*.700 species of angiosperms), different languages and dialects of the 42 tribes, persistent need for traditional medicine and well advanced oral tradition which results in knowledge being widely held in a community (Kokwaro,1983).

Catha edulis is primarily consumed for its psychostimulating effects. However,little is known on the ethnomedicinal uses of this plant. In addition, the few studies that have been carried out on medicinal uses of this plant have often yielded conflicting information. For instance, Mwenda *etal.*(2003) reported that khat could be of use in the treatment for erectile dysfunction. In his experiment with olive baboons, he found that khat chewing increased testosterone hormone levels, libido and erection. Also, WHO (2006) and Kokwaro (2009) have reported the use of khat in treatment of erectile dysfunction. On the contrary, Islam*et al*. (1990) reported that chewing of khat causes loss of libido and seminal output on the chewers. Nyongesa*et al*. (2008) showed that low doses of khat extract increases testosterone hormone level while high doses cause reduction of the hormone level.

Jorgensen and Kaimenyi (1990) reported that the oral hygiene status of khat chewers is better than that of non-khat chewers and that khat chewing is not detrimental to periodontal health. In addition, Nezar and Mohammed (2010) reported that khat chewing may have anti-gingivitis properties and decreases susceptibility to periodontitis. On the contrary, Astalkie*et al*. (2015) indicated that long-term khat chewing negatively affects the oral health. Kokwaro (2009) reported the use of khat in treatment of stomach upset. On the contrary, Nigussie*et al*. (2013) reported a close association between khat chewing and gastrointestinal disorders. These results are not only interesting but also conflicting. Thus, there is a need for a thorough and systematic investigation of the ethnomedicinal uses of *C.edulis.*

2.6 Numerical taxonomy

Taxonomy is concerned with discovery, description and classification of species. Correct description and delimitation of species as fundamental units of systematics is very important for ecological and evolutionary studies. Morphological characters have traditionally been the most important criteria in making taxonomic decisions and remains so despite advances in modern methods of plant taxonomy including use of molecular markers (Crawford, 2000). There are a lot of problems with the species delimitation between closely related taxa due to a great and often continuous morphological variability (Fjeelhem *et al*., 2001). Numerical taxonomy offers solution to such problems.

Numerical taxonomy, also termed as morphometrics deals with grouping by numerical methods of taxonomic units into taxa on the basis of their character states (Sneath and Sokal, 1973). Cluster analysis and principal component analysis are two techniques commonly used in numerical classification. Cluster analysis produces a hierarchical classification of entities (taxa) based on the similarity matrix. It thus provides a logical means of expressing the relationship existing between taxa (Mubo *et al*., 2000). Numerical taxonomic studies are important for discovering and documenting new morphological characters and character states, and many attempts have been made in this regard for understanding phenetic relationships in different groups of plants (Mulumba and Kakudidi, 2010; Deshmukh, 2011; Rahman and Rahman, 2012).

2.7 Chemotaxonomy

Chemotaxonomy of plants is defined as scientific investigation of the potentialities of chemical characters for solving problems of plant taxonomy and plant phylogeny (Hegnauer, 1965). Evidence of chemical variation has been utilized in classification purposes by mankind since ancient times based on traditional knowledge of certain blatant plant characteristics such as edibility, taste, colour, smell, and medicinal value (Mannheimer, 1999).

Bhargava *et al*. (2013) argues that the systematic status of a taxon and its phylogeny should be properly assessed after putting into consideration the external morphological study and other lines of evidence from phytochemical, molecular, anatomical and embryological studies. Thus, chemical variation in plants is one of the most important tools that can help in systematic and phylogenic treatment of plant taxa. Mannheimer (1999) further argues that chemical variation is of taxonomic value as it aids in: Confirmation or support of putative classifications from other sources of taxonomic characters such as morphology; resolution of problems where relationships based on other evidence are ambiguous or conflicting; providing evidence to suggest more natural positioning of anomalous taxa, as well as to separate taxa; detection of confirmation of hybridization as well as providing additional on/off characters for numerical taxonomy by their presence or absence in taxa.

Secondary metabolites of plants such as alkaloids, flavonoids among others have been highly useful taxonomically (Stuessy, 1990). Many attempts of chemotaxonomy have been made in different groups of plants and shown to be a reliable method of delimiting various plant taxa (Ayesha*et al*., 2009; Crockett and Robson, 2011).

2.7.1 Chemistry of *Catha edulis*

To date, alkaloids of *C.edulis* have been the centre of interest to chemists and pharmacologists. *C.edulis* contains two classes of alkaloids namely: Phenylalkylamines (cathinone, cathine, merucathine, norephedrine, pseudomerucathine, norpseudoephedrine) and cathedulins. Examples of cathedulins include: K1, K2, K6, K11, K12 and K15 isolated from Kenyan khat, E2, E3, E4, E6 and E8 isolated from Ethiopian khat and Y7, Y8, Y9, Y10 isolated from Yemen khat (Crombie,1980). Cathedulins arebelieved to bemore than 62 in number (WHO, 2006; Balint, 2012). Generally, it is believed that there are more than 40 alkaloids, glycosides, tannins and terpenoids in *C.edulis* (Elmi, 1983). Gonzalez*et al*. (1986) isolated four compounds from the root bark of *C.edulis* namely: iguestrin, celastrol, tingenone and pristimerin.

The earliest chemical analysis of *C.edulis* was carried out by Fluckiger and Gerock (1887) who analyzed the leaves of *C.edulis* for caffeine. No caffeine was detected but an alkaloid of undetermined structure was isolated and given the name katin. Stockman (1912) isolated three alkaloids which include cathine, cathinone and cathidine but he did not characterize them structurally. Paris and Moyse (1958) detected three to six alkaloids depending on the method of extraction. They suggested that one of the alkaloid was ephedrine. Karawya *et al*. (1968) isolated three alkaloids in addition to cathinone, cathidine and eduline. No chemical structures were proposed for the isolates.

From 1972 onwards, the United Nations Narcotic Laboratory embarked on comprehensive chemical investigations of *C.edulis*. The sample for the isolation of the active ingredient was fresh and dried leaves of *C.edulis* species from Meru, Kenya (Adugna, 2009). Several chemists were recruited and vast financial resources were allocated. Chemical investigation culminated with isolation and characterization of a new compound (-)-aminopropiophenone given the name cathinone (United Nations, 1975). The absolute configuration ofcathinone was afterwards described by Schorno and Steinegger (1978) and the structure of the compound confirmed through synthesis. Schorno and Brenneisen (1978) showed that the phenylalkylamine cathinone is the major alkaloid in *C.edulis* accounting for 70% of total phenylalkylamines present in *C.edulis*. Cathinone is known to be the major active ingredient in khat concentrated mainly in young leaves. Cathinone has almost similar structure and effects to amphetamine thus it was termed as natural amphetamine (Mathys and Brenneisen, 1993). This compound is unstable and undergoes transformation into a more stable compound called cathine within 48 hours of harvesting of plant material (Szendrei, 1980; Brenneisen and Gesshusler, 1985).

According to Kalix and Braenden (1985), cathinone and cathine are found in the ratio of approximately 4:1 in the leaves of *C.edulis*. The action of cathinone and cathine on the reuptake of epinephrine and norepinephrine, neurotransmitters, has been demonstrated in laboratory animals showing that one or both of these chemicals cause the body to recycle these neurotransmitters more slowly, resulting in the wakefulness and insomnia associated with khat. Research has also shown that cathinone is responsible for the feelings of euphoria (AI-Motarreb *et al*., 2002a).

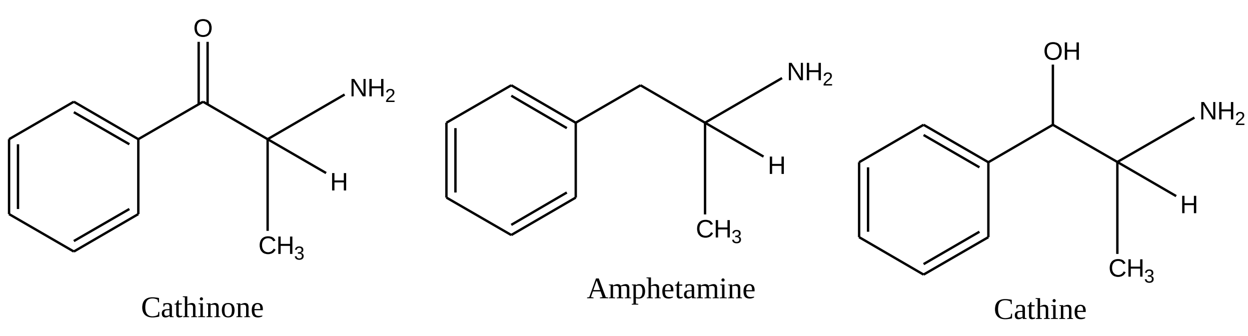

Figure 2. 1: The structure of cathinone, amphetamine and cathine (Nyongesa and Onyango, 2010)

Knowledge of organic components of plants is desirable, not only for the discovery of therapeutic agents but also because such information can be of value in disclosing new sources of such economic materials such as tannins, oils, gums, precursors for synthesis of complex chemical substances and so on (Okach *et al*.,2013). The previous chemical studies on *C.edulis* were incoherent since they never took into account the distribution of such compounds across various parts of traditional varieties (such as *muguka, miraamiiru* etc) of *C.edulis* reported to have quite different properties and pharmacological action (Carrier, 2007; Al-Thobhani *et al*., 2008). Most of the research was done on leaves of *C.edulis*. In Kenya,the twigs of*C.edulis* from Meru are the consumable parts with the leaves being discarded compared to Embu wherebythe leaves of *C.edulis* are the main source of psychostimulating effects. Chemical analysis of different consumable parts of *C.edulis* would not only reveal the possible differences in quality and quantity of phytoconstituents in these parts but can also explain the reason behind preference of leaves to stems/twigs in some cases and stems/twigs to leaves in other cases.

2.8 Pharmacological and physiological effects of *Catha edulis* on humans

The chewing of *C. edulis* material has been more than often associated with negative impact to consumers. However, a few individuals have highlighted some potential benefits of chewing *C.edulis* material. For instance, Darby *et al*. (1959, cited in Lemessa, 2001) points out the potential source of nutrients such as proteins, fibre, ascorbic acid, niacin, riboflavin, beta-carotene, calcium and iron from consumable fresh parts of *C.edulis*. Margetts (1967) argued that consumption of *C.edulis* does not have much adverse effects to the general populace. Nevertheless, Szendrei (1980), Hassan*et al*. (2007), Al-Dubai *et al*. (2006),Cox and Rampes(2003)among others have given quite a number of adverse effects of chewing *C.edulis* material to human beings as summarized in the table 2.1.

Table 2. 1: Pharmacological and physiological effects of chewing *Catha edulis*

Part/system of the body affected	Health effects	Reference(s)
Central nervous system	Dizziness, fine tremor, insomnia, headache	Kalix, 1984;Hassan *et al*.,2007
Obstetric effects	Stillbirths, low birth weight, impaired lactation	Szendrei, 1980.
Metabolic and endocrine effects	Hyperthermia, perspiration, hyperglycemia	Cox and Rampes,2003; Al-Dubai *et al*.,2006
Ocular effects	Blurred vision, mydriasis.	KhattabandAmer (2005);Nencin *etal.*,1984
Genito-urinary system	Urinary retention, impotence, libido change.	Szendrei, 1980; Cox and Rampes, 2003.
Psychiatric effects	Lethargy, irritability, psychotic reaction, depression.	Pantelis *et al.*, 1989.
Cardiovascular system	Hypertension, pulmonary oedema,vasoconstriction,infarction	Hassan *et al.*, 2000; Almotarreb, 2002.
Respiratory system	Tachypnoea, bronchitis.	Cox and Rampes,2003
Gastro-intestinal system	Dry mouth, dental caries, periodontal disease, chronic gastritis, constipation.	Elmi, 1983; Cox and Rampes, 2003, Al-Hebshi *et al.*, 2005.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out in Meru and Embu counties of Kenya which were chosen due to the extensive cultivation of *C.edulis* species as well as socio-economic bearing of this plant species on the livelihoods of residents of the two counties.

3.1.1Meru County

Meru County is found in eastern region of Kenya, approximately 225km northeast of Nairobi. It covers an area of 6,936km^2.Meru county shares its border with five other counties which include: Isiolo to the North, Nyeri to the South West, Tharaka-Nithi to the South west and Laikipia to the West (Figure 3.1).

The origin of the word Meru is believed to come from the Maasai people who referred to Tigania and Imenti forests as the Mieru forests or simply the 'Quiet Forests'(Nyaga, 1997). Maasais are also believed to have used the term *Mieru* to name any tribe which could not understand their Maa language. Meru County is made up of nine constituencies which include Igembe South, Igembe Central, Igembe North, Tigania West, Tigania East, North Imenti, Buuri, Central Imenti and South Imenti. According to 2009 census, Meru County has a population of approximately 1,365,301. The main people who live in Meru comprise sub tribes of Ameru community which include the Imenti, Tigania and Igembe(Fadiman, 1994).The major towns in Meru County include Meru, Maua and Nkubu.Meru town is the commercial and administrative centre of Meru County. It is one of the economically viable towns due to thriving *Catha edulis* trade.

The climate of Meru is described as cool and warm, with temperatures ranging between 16^{o}C during the cold season and 23^{0}C during the hot-warm season. Meru receives an average rainfall of between 500mm and 2600mm each year. Agriculture is the main economic activity in Meru County. Most people in this county are involved in subsistence farming where they grow common foods such as maize, beans, sorghum, millet, cabbages and fruits. The county is well known for its wide scale growing of *C.edulis* a lucrative cash crop for the locals. *C.edulis* species is mostly grown in Maua, Igembe and Tigania and fetches millions of shillings in the export market for its farmers (Carrier, 2007).

3.1.2 Embu County

Embu County is located approximately 120 km northeast of Nairobi towards Mt Kenya. It covers an area of 2,818km^2. According to 2009 census (Kenya Bureau of Statistics, 2009), Embu County has a population of approximately 516,212 individuals. The county is made up of four constituencies which include: Manyatta, Runyenjes, Gachoka and Siakago. The major towns in Embu County include Embu which is an administrative and commercial centre of the county, Siakago, Kiritiri, Ishiara and Kanyuambura. Embu County borders six other counties which include Tharaka Nithi to the North, Kitui to the East, Machakos to the South, Murang'a to the South West and Meru to the North West (Figure 3.1). Embu occupies among the most main fertile lands in the Kenyan highlands, with its weather favorable for a variety of agricultural activities.

The major economic activities in Embu County include food crop farming such as maize, beans, yams, cassava, millet, sorghum, bananas, and arrowroots among others. There is also the farming of cash crops such as coffee, tea, pyrethrum and a 'variety' of *Catha edulis* locally known as *muguka* which is now becoming very popular especially in Mbeere region. Livestock keeping is also popular here. The major attractions in Embu County include the Seven Folks Dams for power generation, the Karue Hill which towers high along the Embu/Meru highway, two main waterfalls that join to form the Ena River that meanders to encircle the Karue Hill, Kirimiri hill which is home to a wide range of wildlife.

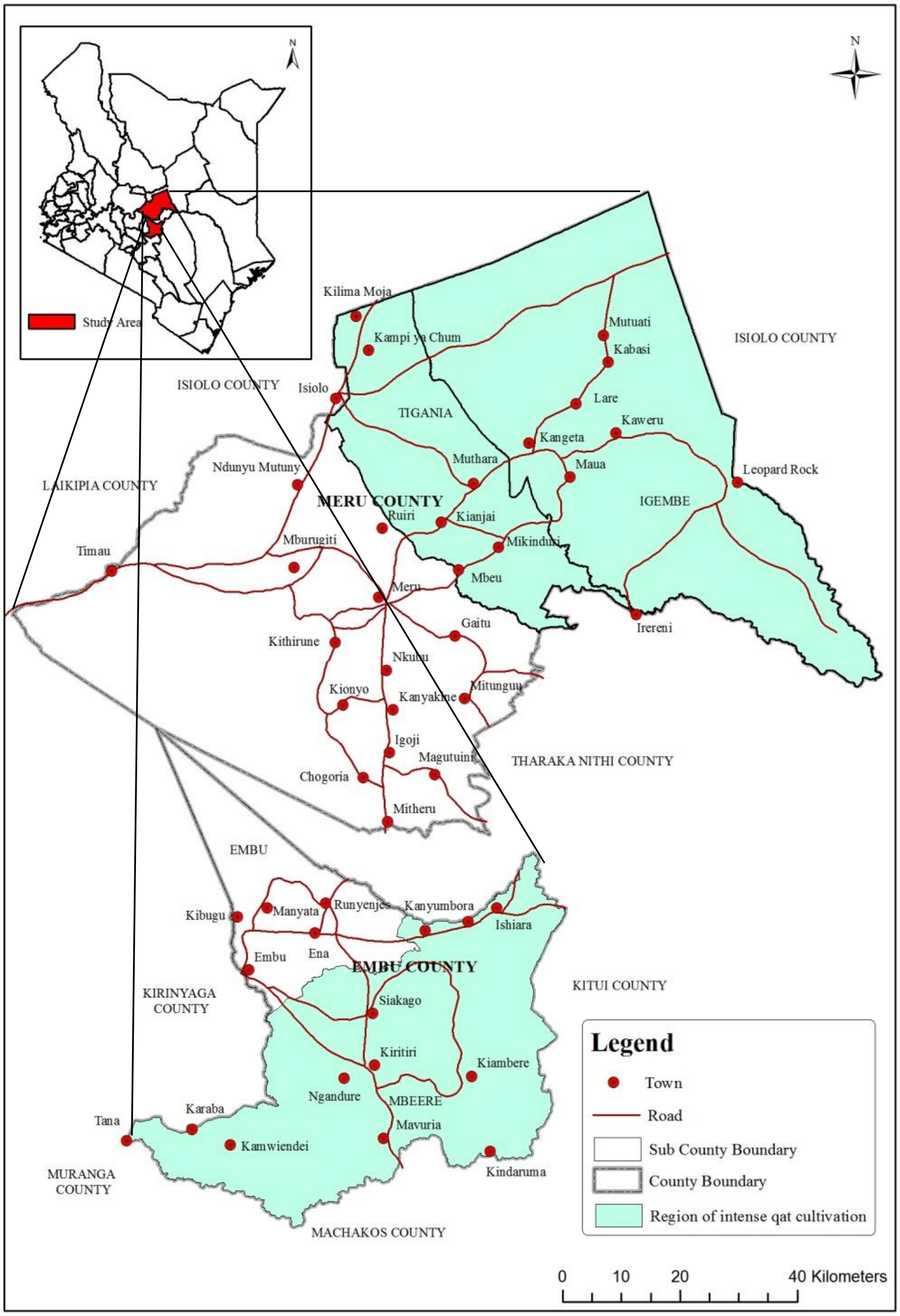

Figure 3. 1: A map of Meru and Embu counties. Inset:map of Kenya (Source:Survey of Kenya,2011)

3.2 Ethnobotanical data collection

3.2.1 Reconnaissance (Initial Contact with Respondents)

A field survey was done prior to data collection during which a list of farmers of *C.edulis* as well as herbalists in Meru and Embu counties respectively was prepared with the assistance of administrators (Chiefs, Assistant Chiefs) and the general public of the various locations where they are found in these Counties (Owuor, 1999).

3.2.2 Selection of Key Informants

Forty two key informants (32 males and10females) aged between 45 and 84 years were identified for interviews in Mbeere,Tigania and Igembe.A combination of snowball and purposive sampling techniques were used to identify the key informants. This involved identifying a few informants(farmers and consumers) and using these initial contacts to identify more informants resulting in a snowball sample of forty-two informants This procedure is widely used in ethno knowledge studies to get information from hidden populations, which are difficult for researchers to access (Gakuubi and Wanzala, 2012; Salganik and Heckathorn,2004)

The purposive sampling technique ensured that only key respondents with the desired qualities and quantities of information on *C.edulis* were selected (Russell*et al*., 2002).A probability random sampling technique would not have been appropriate for this type of socio-cultural set-up, as not everyone sampled randomly would have the required knowledge (Etkin, 1993;Cotton, 1996;Cunningham, 2001).

3.2.3 Collection of Ethnobotanical Information

A semi-structured questionnaire consisting of fifteen questions was used in collecting ethnobotanical data in Meru and Embu counties. The questions required:-(1), location of the interviewee; (2), informant's details (name, sex, age, level of education, occupation); (3), informant's consent agreement; (4), brief history of plant(including its origin); (5), traditional varieties known by the respondent; (6), morphological distinction of each cultivar based on traditional knowledge; (7), stimulating level of each traditional variety relative to other traditional varieties; (8), types of market grades available; (9),differences among grades; (10), propagation method of each traditional variety; (11),medicinal uses of the plant; (12), parts of the plant used; (13), method of crude drug preparation; (14) mode of drug administration; and (15), Prescribed dosage.

3.2.4 Conduction of Interviews

Interviews were conducted face to face with a help of an interpreter and answers filled by the interviewer. After filling of the well-structured questionnaire, discussion with the selected key respondents was held. This was guided exchanges semi-structured by a mental checklist of relevant points to confirm the validity of the information in the questionnaires of other key respondents interviewed earlier.

3.2.5 Collection of Plant Samples

Following a face to face interview with the selected key respondents, a field trip was made to identify and collect the listed plant specimens. Three to five branchlets with flowers and fruits were collected from each individual sampled to provide enough material for phenetic study as well as for herbarium vouchers deposited at the University of Nairobi herbarium as per the procedures in Mulumba and Kakudidi(2010). Only healthy plants were sampled.GPS waypoints of locations where plant samples were collected were recorded in a field notebook.

3.3 Numerical Taxonomy

3.3.1 Selection of Operation Taxonomic Units (OTUS)

Fifteen traditional varieties (named by the farmers in the field) of *C.edulis* were selected for numerical taxonomic study. These included the eleven traditional varieties represented in Table 4.1 plus an unknown type and other three traditional varieties sharing common names.They were collected from different geographical locations designated as *muguk*a B, *Kiraagikiiru* B and *Kiraa gikiiruC.*

3.3.2 Character Scored and Method of Scoring

Characters were selected based on Adansonian principle, that is, many characters should be considered when making taxonomic decisions (Sokal and Sneath, 1973). Certain characters were discarded as research progressed because they proved constant throughout the group, were difficult to assess accurately or were unsuitable for rapid and accurate scoring (Mubo *et al.*, 2004). A set of thirty-three characters representing the plant habit, vegetative and floral characters and comprising qualitative and quantitative characters were generated from field observations and thestudy of fresh collections. Out of these, twenty-three were qualitative while ten were quantitative. All measurements in each sample were carried out ten times and their averages recorded. The character state “shrub” referred to individuals without a clear trunk and branching from or close to the base while the character state “tree” referred to individuals with a clear trunk. Leaf length was taken as lamina length while leaf breadth was taken at the widest point of the leaf. An ocular lens mounted on a dissecting microscope was used to estimate the diameter of the disc. The Royal Horticultural Society (2001) standard colour chart was used in colour scoring. The latest description of *C.edulis* given in FTEA (1994) was highly helpful in making observations of fresh collections both in the field and in the laboratory. The thirty-three characters and their states were entered into Microsoft excel and coded into binary states before being subjected to hierarchical cluster analysis. All the 33 characters were majorly derived from fresh collections contrary to the normal procedure of using herbarium material. Marhold(2011) proposed sampling individuals in the field as opposed to use of herbarium material because intra-population variation might be hidden as some characters are not preserved on herbarium specimens. The table overleaf, Table 3.1shows the characters and their character states used in numerical taxonomy. The codes employed to represent the character states in the data matrix are given in parenthesis following each state.

Table 3. 1: Character/Character States used in Numerical Taxonomic Analysis of *Catha edulis*

No.	Characters	Character states
1	**Habit**	**Tree (1); Shrub(0)**
2	Bark colour	White(1); grey(0)
3	Branches	Pendulous(1); erect(0)
4	Branchlets	Narrows and round(1); thick and flattened(0)
5	Nodes	Swollen(1); not swollen(0)
6	Leaf pairs	4-8(1); 10-20(0)
7	Colour of young twigs	Crimson or red(1); pale green on abaxial surface, red on adaxial surface/pale green(0)
8	Leaf arrangement	Opposite(1);opposite on old branches, alternate on young twigs(0)
9	Colour of young leaves	Dark -red, margin purplish(1); red or light red(0)
10	Laminar shape	Lanceolate or ovate-lanceolate (1); oblong or oblong-elliptic(0)
11	Margin type	Crenate(1); crenulate(0)
12	No. of marginal teeth	50-64(1);28-47(0)
13	Leaf length	>10(1);5.8-7.5(0)
14	Leaf width	3.1-4.7(1);1.8-2.7(0)
15	Apex shape	Retuse (1); acute or sub-obtuse or obtuse (0)
16	Leaf symmetry	Symmetrical(1);asymmetrical(0)
17	Leaf base	Cuneate(1);attenuate (0)
18	Petiolar shape	Flattened on upper surface(1); terete (0)
19	Petiolar length	>6.0(1);3.1-5.3(0)
20	Petiolar colour	Pale green(1);Purple(0)
21	Ovary shape	Narrowly ovoid(1);spherical(0)
22	Disc diameter	1.0-1.5(1);0.5-0.9(0)
23	Orientationofpeduncle	Spreading at right angles(1);not spreading at right angles(0)
24	Petal colour	White(1); pale white(0)
25	Petal length	0.6-0.9(1);1-1.5(0)
26	Peduncle length	6-12(1); 2.5-5.7(0)
27	Petal margin	Entire(1); slightly clawed (0)
28	Fruit shape	Ellipsoidal(1); oblong(0)
29	Fruit colour	Red(1); brown(0)
30	Fruit length	3.5-5.2(1); 5.9-9.4(0)
31	Seed colour	Red(1); light brown o dark(0)
32	Aril length	4.9-6.8(1); 2.5-4.5(0)
33	**Seed length**	**1.2-2.7(1); 3.0-3.7(0)**

3.4 Chemotaxonomy of *Catha edulis*

3.4.1 Collection of Plant Material

Khat was bought from farmers in the locations previously identified during ethnobotanical survey. The samples included those from khat trees/shrubs that had not been exposed to chemical spray. Khat sample from each of the traditional varieties mentioned during ethnobotanical survey was harvested, tied into bundles and wrapped with wild banana (*Ensete ventricosa*) fibers in case of khat from Meru county or common banana fibres(*Musa* spp.) in case of khat from Embu County. These were packed in small polythene bags and immediately transported to the laboratory while fresh for chemical analysis.

3.4.2 Preparation of Crude Extracts for TLC Analysis

Thin Layer Chromatography (TLC) is a rapid analytical tool which can be used to determine the components in a mixture (Muchena, 2009). In this study, the standard method developed by Lee *et al*. (1995) for rapid TLC analysis of cathinone and cathine in *C.edulis* was applied. Both leaves and stems were analyzed separately. Young stems of fresh khat material of each traditional variety of *C.edulis* were rendered leafless. The leaves were kept separate. Young leaves preferably those towards the tip of the stem were used for chemical analysis.

The tip of the active growing part of each *C.edulis* stem for each variety was chopped off. Each stem was cut into small pieces (about 1mm) and prepared for extraction. The young leaves were prepared in the same way. A15 g portion of young stems (or leaves) of *C.edulis* was mixed with 30 ml of methanol and ground properly in a mortar. The suspension was transferred into a test tube with a stopper and shaken manually for 10 minutes. The mixture was filtered through a Buchner funnel and the filtrate concentrated in a rotary-evaporator.The concentrated extract was filtered again.

Five drops of 0.01M H_2SO_4were added to the filtrate, until it acquired a brownish hue.This was done to re-suspend and acidify the crude extract. This acidified extract was extracted into dichloromethane by solvent-solvent partitioning (three times) to remove neutral organic compounds as well as remaining plant solids. A small amount of saturated sodium bicarbonate was used to basify the aqueous layer. The now light green aqueous suspension containing free khatamines was extracted again with dichloromethane. The same procedure was followed for the extraction of leaves.

3.4.3. Preliminary Analysis of Khat and Comparison of the Separation Efficiency of the Two Solvent Systems.

The methanol extraction followed by acid-base extraction was shown to be a time consuming process especially where large samples were to be analyzed and therefore, in the present study, a quicker, more direct method was tried on four fresh samples before the standard procedure was applied to determine whether khatamines could be detected after TLC Analysis and hence act as a substitute for this time consuming and tedious process.

In this case, TLC analysis was done immediately after methanol extraction of fresh khat material on silica gel plate (matrix, 5cm x 10cm). Five microlitres of the filtrate were applied band wise (5-mm line) to a silica gel plate (matrix, 5cmx10 cm). This was done in duplicate. Various organic solvents such as mixtures of n-hexane, ethyl acetate and acetone were used as solvent systems.

The same procedure was repeated using $EtOAc/MeOH/NH_3$ (8.5:1.0:0.5) and $CH_2Cl_2/MeOH$ (9:1) as solvent systems which have been previously used in analysis of khatamines of *C.edulis.* The plates were then dried under room temperature prior to visualization under UV light (254nm), sprayed with ninhydrin reagent (0.3g in 100ml nbutanol, 3ml conc.acetic acid) and heated at 110^{o}C for two minutes as recommended by Leihmann*et al*. (1990).

3.4.4 Determination of the Decomposition Rate of the Observed Compound

This was done to help know how long the fresh material of *C.edulis* could be stored after collection from the field before TLC analyses were carried out since methanol extraction followed by acid-base extraction of khatamines proved to be a long and tedious process if many samples were to be analyzed. It was also a way of determining whether cathinone was present in the samples as it is the only khatamine known to be very unstable. Szendrei, (1980) and

Brenneisen and Gesshusler, (1985) showed that cathinone is very unstable and decomposes within 48 hours after khat is harvested.

After extraction of four fresh samples, TLC analysis was made subsequently after every two hours except where the extracts were left overnight and analysis continued the following day. The visible spot continued to decrease in size until complete decomposition was observed.

3.4.5 Analyses of Fresh Leaves and Young Stems for Khatamines

After carrying out the preliminary analyses, 11 fresh samples of *C.edulis* (6 from Meru County and 5 from Embu County) collected the same day were extracted by acid-base extraction method and analyzed simultaneously for the presence of khatamines in fresh young leaves and stems of *C.edulis*. The standards were not available for the identification of the compounds present in plant extracts.The solvent system used was CH_2Cl_2/MeOH (9:1).Ninhydrin reagent was used to detect khatamines of *C.edulis* as they contain amine group (-NH_2) in their structure (Fig 2.1) which forms a complex with ninhydrin resulting in colour change.

3.5 Data Analysis

Data was entered into spreadsheet and checked for correctness before being subjected to statistical analysis. Ethnobotanical data was analyzed by use of descriptive statistics (Gakuubi and Wanzala (2012). The morphological data matrix was scored using binary matrix. Dissimilarity matrix was prepared based onthe data matrix. Cluster analysis was performed using UPGMA (Unweighted Pair Group Method with Arithmetic mean) and a dendrogram was constructed to show the relationship among the traditional varieties studied (Sneath and Sokal, 1973). R_f values were calculated after TLC analysis (Harborne, 2002). All the analysis was carried out using IBM Statistical Package for Social Sciences (SPSS) version 20.

CHAPTER FOUR

RESULTS

4.1 Ethnobotanical Survey

4.1.1 Traditional Naming of *Catha edulis* in Meru and Embu Counties.

The people of Ameru and Aembu communities of Kenya have traditionally developed an elaborate system of classifying plants based on several criteria such as locality, age, type, and edibility. Traditional varieties of *C.edulis* have been classified based on several criteria such as age of trees, morphology, and stimulating effects of various traditional varieties. *Miraa* (singular *muraa*) is a common vernacular name of harvestable young twigs from *C.edulis*.It was observed that the people of Meru classified both the *miraa* trees and the young twigs harvested. Thus names such as *kangeta* and *giza*were commonly encountered in miraa market which denoted grades of *miraa* rather than reference to a particular tree type. They were graded based on the length of the twigs harvested. For instance, *kangeta* referred to twigs of *miraa* of length 20-30cm.Anything shorter than this was referred to as *giza* (*kisa*). *Mbaine* referred to any*miraa* tree dating back to over 300 years ago rather than to any particular traditional variety of *C.edulis*. Younger trees of *miraa* were locally known as *Mithairo*. The table below gives the various common names (traditional varieties) of *C.edulis* found in Meru and Embu County.

Table 4. 1: Traditional Varieties of *Catha edulis* collected in Meru and Embu counties

S/n	Local name	County	Latitude	Longitude	Altitude(ft)	Voucher
1	Gitu	Embu	S0.65746	E37.56912	3793	JK2014/09
2	Kibwe	Embu	S0.62732	E37.54423	3822	JK2014/11
3	Kigwe	Meru	N0.28766	E37.92004	5863	JK2014/02
4	Kilantune	Meru	N0.13227	E37.74410	4993	JK2014/03
5	Kiraa gikiiru/Asili	Meru	N0.28596	E37.92127	5837	JK2014/01
6	Kithaara	Meru	N0.13507	E37.74546	4882	JK2014/04
7	Muchuri	Meru	N0.13523	E37.74356	4938	JK2014/05
8	Muguka	Embu	S0.62800	E37.54269	3757	JK2014/06
9	Mugumo	Embu	S0.65753	N37.56936	3806	JK2014/12
10	Mugwathingi	Embu	S0.62740	E37.54428	3825	JK2014/08
11	Muti-mutiri	Embu	S0.65746	E37.56931	3802	JK2014/07

4.1.2 Identification of Traditional Varieties of *Catha edulis* by the People of Meru and Embu counties

The Ameru and Aembu people of Kenya have developed a traditional classification system of *C.edulis* (Table 4.2). Some of the characters used by locals were not morphological hence making it difficult to identify them in the field. Nevertheless, those differences were very important to the farmers when it came to grading khat for sale.

Table 4. 2:Traditional Description of *Catha edulis* by Meru and Embu People

S/n	Traditional variety	County	Key morphological characteristics	Khat features and associated physiological effects
1	Kiraa gikiiru/asili	Meru	Young twigs dark-red in colour.	Produces softer khat, tastes nice, powdery when chewed, no negative physiological effects like delirium, insomnia etc. Most preferred variety.
2	Kigwe/Kiraa gikieru	Meru	Leaves broad, young twigs red on abaxial surface and light green on adaxial surface.	.Bitter taste, highly potent with negative physiological effects if chewed in excess amount such as delirium, insomnia, and formication (feeling of insects crawling over the body).
3	Muchuri	Meru	Evergreen tree, young twigs red in colour.	Highly productive in all seasons. Commonlyfound in Tigania region of Meru County.
4	Kithaara	Meru	Leaves narrow, profuse branching, branches flexible.	Sugary taste, potent but notas much as *kigwe*, commonly found in Tigania region of Meru county.
5	Kilantune	Meru	Young twigs red.	Tasteless, less preferred.
6	Muguka	Embu	Bushy shrub, bitter taste, red shoots, branches inflexible.	Highly potent with effects being felt immediately after masticating a few leaves most preferred because it's cheaper and has strong inebriating effects.
7	Muti-mutiri	Embu	Bushy shrub, shoots light greenish, tends to grow tall.	Not as potent as *muguka*.
8	Mugumo	Embu	It is referred by this name due to its evergreen nature even in drought just like the fig tree. Shrub	It also breaks easily. It has moderate potency level when chewed. Not preferred by consumers.
9	Kibwe	Embu	Leaves oblong, shoots light greenish.	less preferred by consumers
10	Mugwathingi	Embu	Leaves large, shoots light greenish to purplish.	Less potent than *muguka*, less preferred.
11	Gitu	Embu	Leaves large, shoots light greenish.	Less preferred, less potent than *muguka*.

4.1.3 Ethno-medicinal uses of *Catha edulis* in Meru and Embu Counties

A total of thirteen diseases/illnesses were reported to be treated using *C.edulis* in Meru and Embu counties. A summary of the medicinal uses and their percentage use per county is given in Tables 4.3 and 4.4 respectively.

Table 4. 3: Ethnomedicinal uses of *Catha edulis* in Meru and Embu countie

S/N	Disease/ill-health condition	County	Parts used	Method of drug preparation	Mode of administration
1	Helminthiasis	Embu	Leaves	chewing fresh material	Oral
2	Heartburn	Meru	Leaves	chewing fresh material	Oral
3	Stomach upset	Meru	Leaves	chewing fresh material	Oral
4	Dry coughs	Meru& Embu	Stems and leaves	chewing fresh material	Oral
5	Pneumonia	Meru	Leaves	chewing fresh material	Oral
6	Diarrhoea	Meru& Embu	Roots and stems	Boiling fresh material	Oral
7	Generalbody pain	Meru	Stems	chewing fresh material	Oral
8	Erectile dysfunction	Meru	Stems	chewing fresh material	Oral
9	Influenza	Meru	Stems	chewing fresh material	Oral
10	Asthma	Meru	Stems	Chewing fresh material	Oral
11	Toothache	Embu	Leaves	Boiling, then inhaling the vapor through the mouth	Oral
12	Gonorrhea	Meru	Roots	Boiling	Oral
13	Fatigue	Meru& Embu	Stems	Chewing fresh material	Oral

Table 4. 4: Percentage frequency of use for ethnomedicine in Embu and Meru

County	Medicinal uses reported in each/both counties(n=13)	Percentage(%)medicinal use
Meru	8	62
Embu	2	15
Meru&Embu	3	23

Cases of diseases treated by *C.edulis* material werehigher in Meru County (62%) compared to Embu County (15%).Similar medicinal uses in both counties were anti-fatigue,anti-diarrhoea and remedy for dry cough which comprised the remaining (23%). It was noted that most of the key informants in Embu County were not aware of medicinal uses of *C.edulis* other than its use as a psychostimulant.

4.1.4 Parts of the Plant Used

Leaves and young stems were the most common parts of *C.edulis* plant used as a traditional therapeutic measure against common human diseases(See table 4.1). Roots were reported to have scanty usage although cases of treatment of gonorrhea and diarrhoea were reported. It is possible that the medicinal value of leaves and stems might have been realized in the process of trying to acquire the psychostimulating effects of *C.edulis* as these are the most common consumable parts of *C.edulis*.

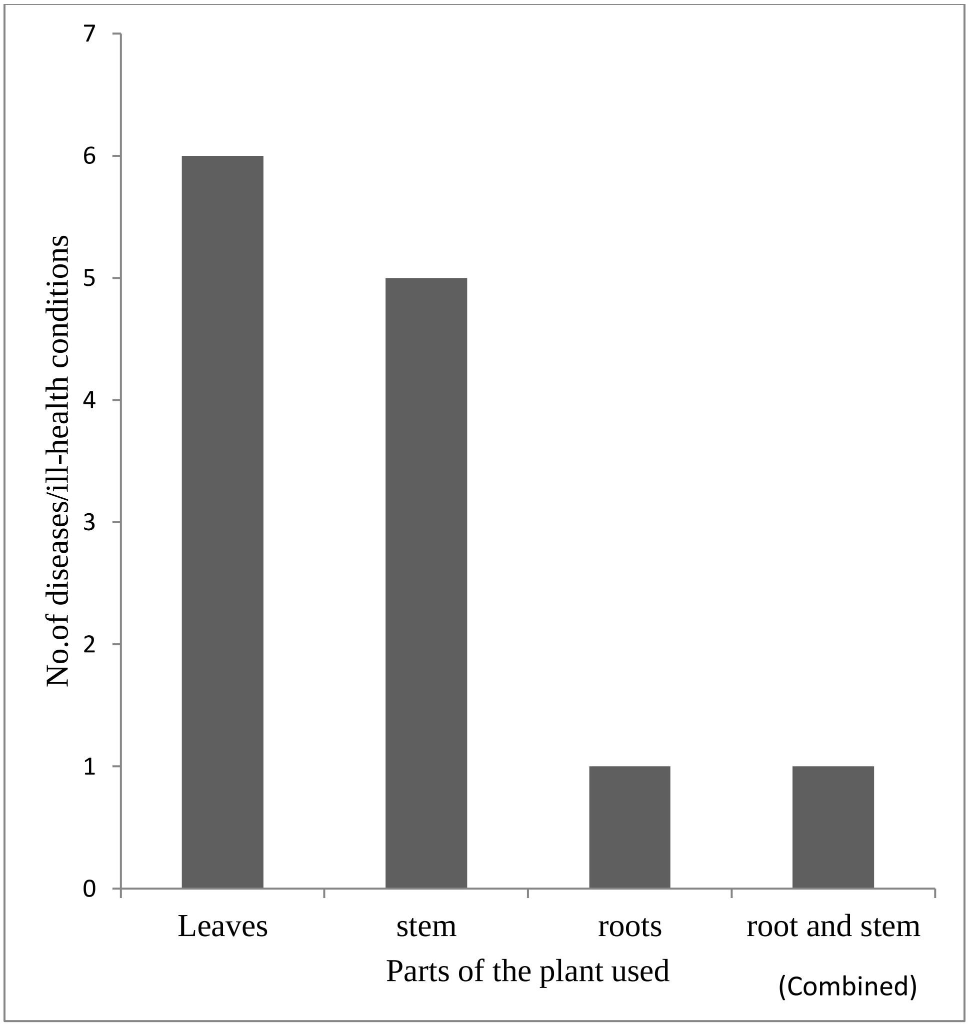

Figure 4. 1: Number of diseases treated and plant parts used.

4.1.5 Method of Drug Preparation and Route of Administration of Prescribed Dosage

Chewing raw khat material was reported to be the main method of acquiring medicinal potential of *C.edulis* although in some cases, boiling of the plant material is utilized. The route of administration of crude extracts was generally oral. In most cases, this occured in the pursuit of stimulating effects of *C.edulis*.There was no precise dosage administered for any given ailment. The amount of khat chewed depended on its availability and individuals concerned.

4.2Numerical Taxonomy

4.2.1 Phenetic Relationship among the Traditional Varieties Studied.

The phenogram revealed two major clusters with the second cluster being subdivided further into two sub clusters (Figure 4.2). The first cluster consisted of all traditional varieties of *C.edulis* sampledin EmbuCounty namely:*Kibwe, mugwathingi*, *gitu,mugumo*, *muti-mutiri*, *muguka A* and *muguka B*. *Muguka A* and *Muguka B* represent a single member as known by the local people but collected in different geographical locations of the same Embu County.

The second cluster consisted of traditional varieties of *C.edulis* collected in different parts of Meru County namely:*Kiraa gikiiru A, Kiraa gikiiru B, Kiraa gikiiru C, kigwe, kithaara, Muchuri, kilantune* and an unknown type). *Kiraa gikiiru* A, B and C were also collected in different geographical locations and represented a single member which was very popular among the local people of Meru as it produced softer khat with a dark tinge commonly known as *miraamiiru* ("black miraa"). Figure 4.2 shows the results of hierarchical clustering using Unweighted Pair Group Method with Arithmetic averages (UPGMA). Figures 4.3 and 4.4 show the habit of the representative members appearing in each cluster.

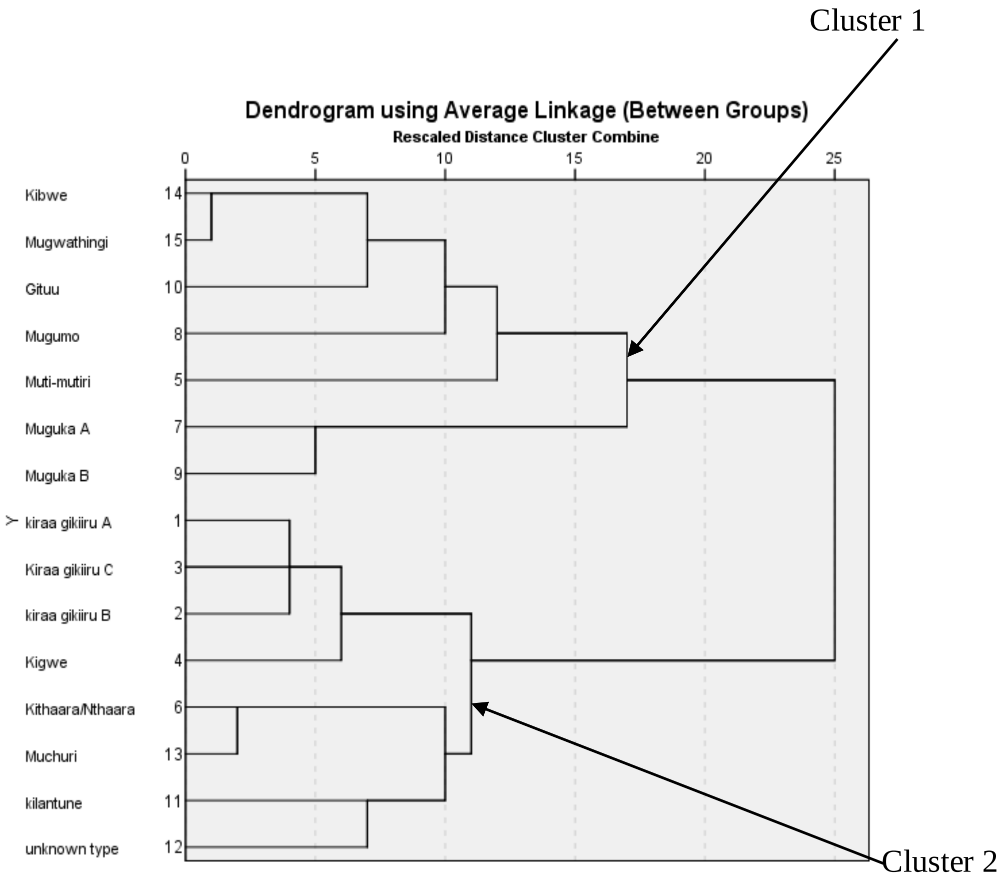

Figure 4. 2: Phenogram showing relationship among the traditional varieties of *Catha edulis.*

This is based on Unweighted Pair Group Method with Arithmetic Average (UPGMA).

.

Figure 4. 3: Shrubs of *Muguka*, a traditional variety in Embu. (Source: Author)

Figure 4. 4: A tree of *Kiraagikiiru,* a traditional variety in Meru. (Source: Author)

4.3 TLC Analysis of Fresh Leaves and Young Stems of Different Traditional Varieties of *Catha edulis*

4.3.1 Preliminary Analysis of Khat with Organic Solvents after Methanol Extraction.

Preliminary TLC analysis of selected samples using organic solvents did not yield any result for khatamines after methanol extraction. Polar compounds seemed stuck at the base. Only chlorophyll and a yellow compound was observed under UVlight (254 nm). Similarly, using CH_2Cl_2/MeOH (9:1) or EtOAc/MeOH/NH_3 (8.5:1.0:0.5) as solvent systems did not work for khatamines after methanol extraction. Perhaps chlorophyll masked the detection of the polar compounds.

4.3.2 Comparison of the Separation Efficiency of the Common Solvent Systems.

The leaves of four traditional varieties (two from Embu County and two from Meru County) were initially used to determine the separation efficiency of the two solvent systems. These were *Muguka*, *Kiraa gikiiru*, *gitu* and *kigwe*.One major compound (R_f value 0.6) was observed under UVlight(254 nm) when leaves of these varieties wereanalyzed repeatedly using CH_2Cl_2/MeOH (9:1) as solvent system. The same was observed when EtOAc/MeOH/NH_3 (8.5:1.0:0.5)was used as a solvent system ($R_f$value0.8). However, after spraying the plate with ninhydrin reagent, a second purple compound near the baseline ($R_f$value0.1)was detected whenCH_2Cl_2/MeOH (9:1) was used as a solvent system but was not detected when the plate was developed using the EtOAc/MeOH/NH_3 (8.5:1.0:0.5)(Figure 4.6).Following this, the subsequent analysis of fresh leaves and young stems of traditional varieties of *C.edulis* relied on the solvent system DCM/MeOH (9:1) as this proved to be a better solvent system than EtOAc-MeOH-NH_3

(8.5:1:0.5).

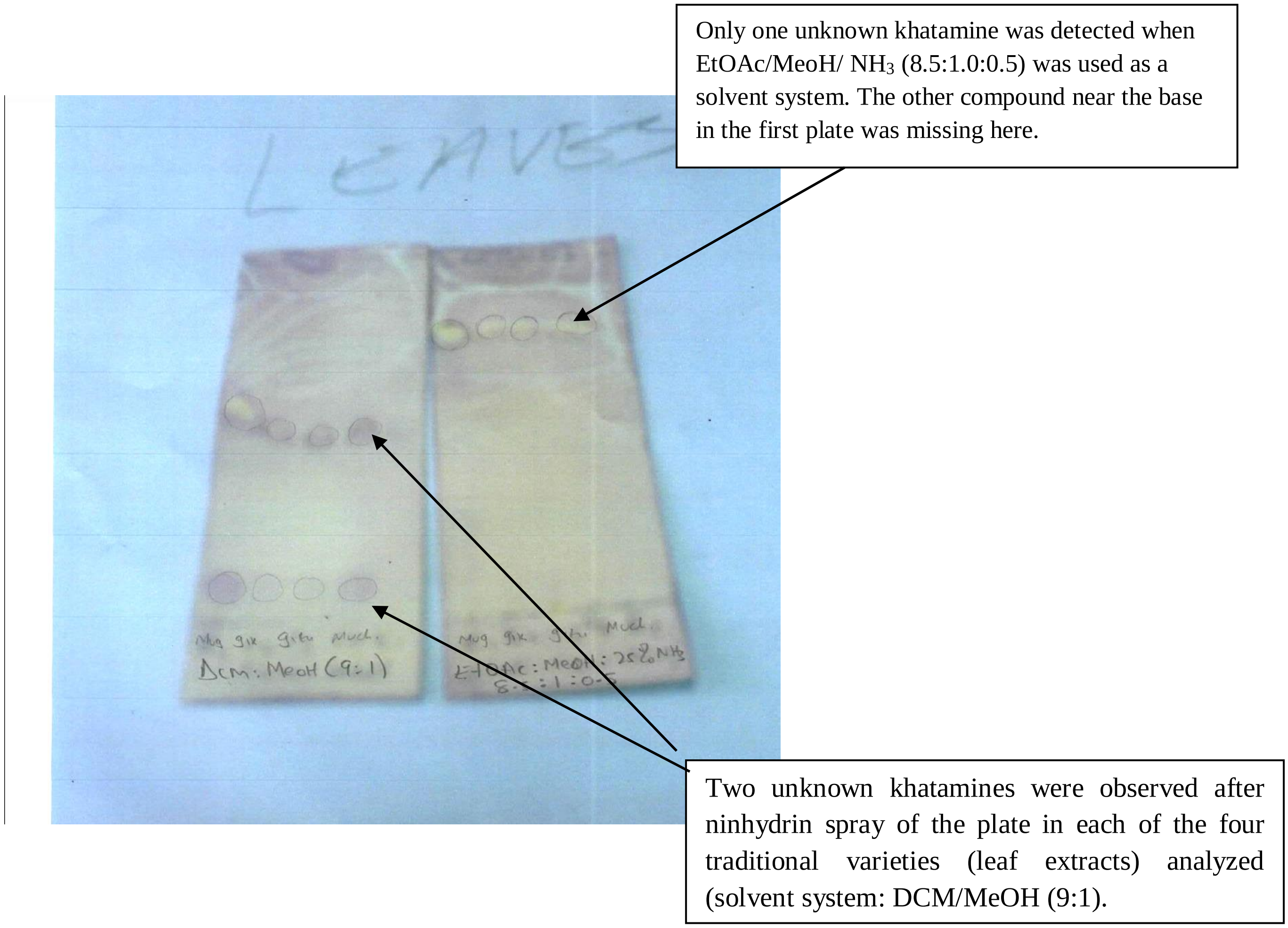

Figure 4. 5: Comparison of the separation efficiency of the two solvent systems.

4.3.3 Decomposition rate of the observed compound

.

The compound which was well visible under UV light (254nm) decomposed after36hours as shown below. Decomposition in this study seemed more rapid after extraction of khat material. What could be seen after decomposition were artifacts of cathinone (Figure4.8).It was clear that fresh material of *C.edulis* could not be stored for a long time if desired results were to be obtained.

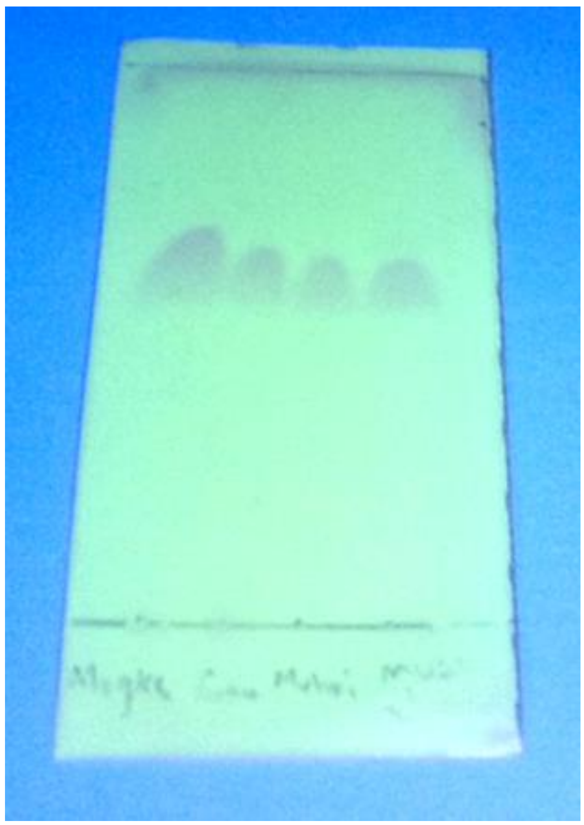

Figure 4. 6: TLC analysis before decomposition (UV light 254 nm)

Legend: Before decomposition, one unknown khatamine was observed under UV light in leaf extracts of all the four varieties analyzed

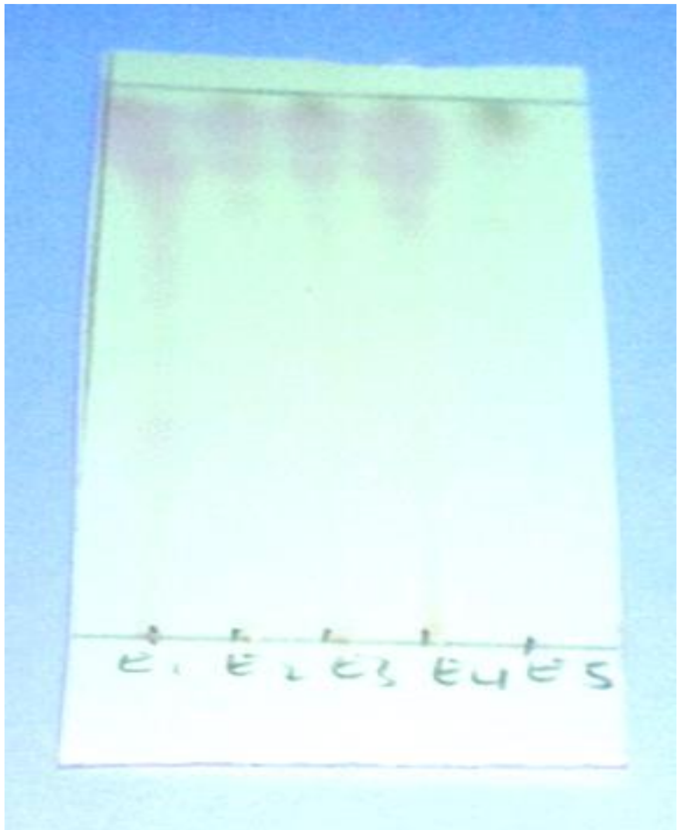

Figure 4. 7: TLC analysis after decomposition (UV light 254 nm)

4.3.4 Analyses of Fresh leaves and Young Stems

The TLC analyses of khat from Meru revealed the presence of two compounds when observed under UVlight (254 nm). One appeared pink ($R_f$value0.5) whereas the other one appeared dark-grey ($R_f$value0.4). After spraying with ninhydrin reagent, the pink compound turned to a burnt orange while the other compound appeared colorless. Another purple compound near the origin (R_f value0.1) was also detected after spraying with ninhydrin. Therefore, in total three compounds were detected in the young stems of all traditional varieties of *C.edulis* from Meru County.

The fresh young stems of khat from Embu County revealed the presence of one compound(R_f value 0.4) when observed under UV light (254 nm).After spraying with ninhydrin, another compound was detected near the origin ($R_f$value0.1) which appeared purple in colour. So in total two compounds were detected in young stems of all traditional varieties of *C.edulis* from Embu County. All the leaves analyzed revealed one major compound (purple, $R_f$value0.6) observable under UV light (254 nm). This compound changed colour to a burnt orange after spraying with ninhydrin reagent. Another purple compound ($R_f$value0.1) near the baseline was also detected after spraying with ninhydrin.Therefore; two compounds were detected in leaves of all the traditional varieties collected in both Meru and Embu counties.

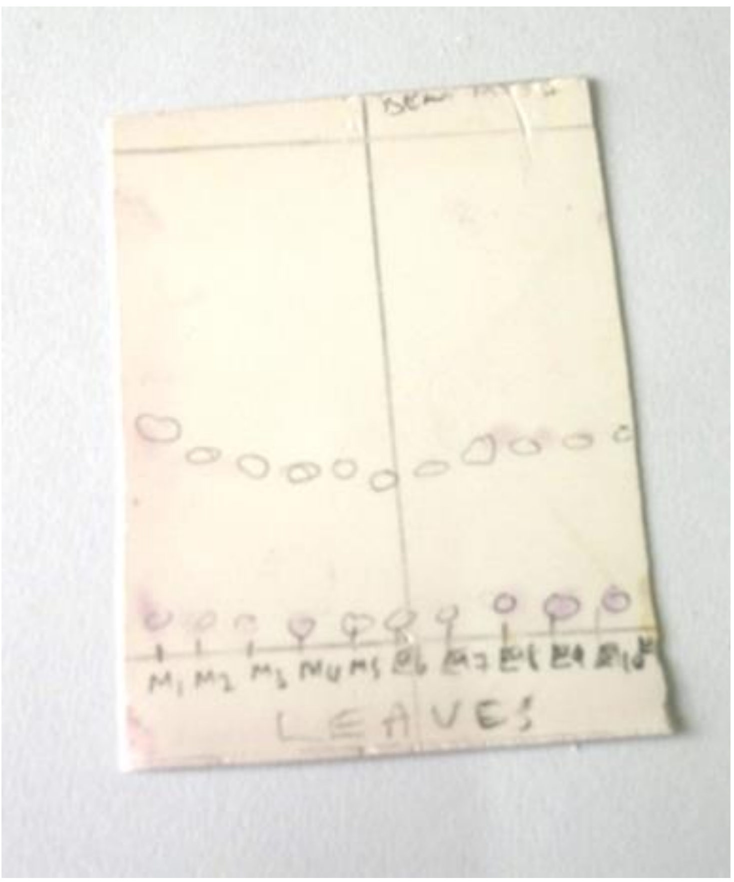

Figure 4. 8: TLC analysis of leaves of 11 traditional varieties of *Catha edulis*

Key:M1=*Kiraagikiiru*;M2=*Kigwe*;m_3=*Kithaara*;M_4=*Muchuri*;M_5=*Kilantune*;E_6=*Muguka*;E_7=*Gitu*;E_8=*Kibwe*;E_9=*Muti-mutiri*;E_{10}=*Mugwathingi*;E11=*Mugumo*(NB:M_1-M_5were from Meru County,E_6-E_{11} were from Embu county)

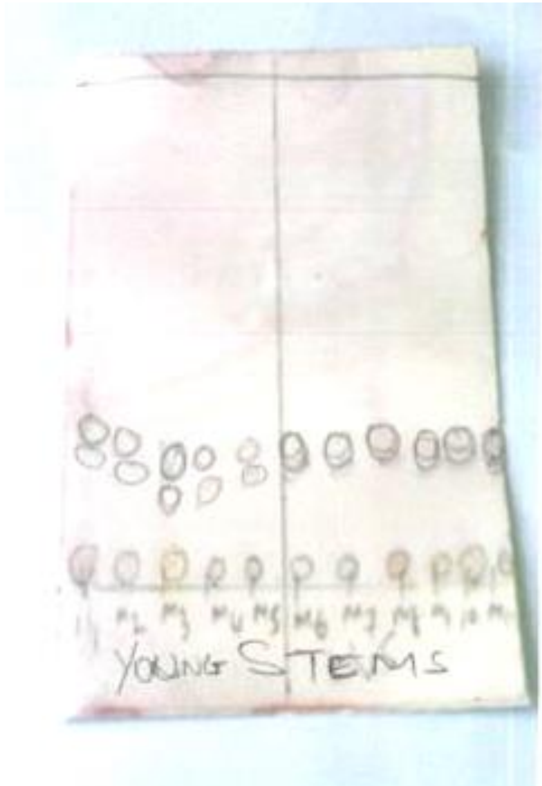

Figure 4. 9: TLC analysis of young stems of 11 traditional varieties of *Catha edulis*.

Key:M1=*Kiraagikiiru*;M2=*Kigwe*;m_3=*Kithaara*;M_4=*Muchur*i;M_5=*Kilantune*;M_6=*Muguka*;M_7=Gitu;M_8=*Kibwe*;M_9=*Muti-mutiri*;M_{10}=*Mugwathingi*;M_{11}=*Mugumo*(NB:M_1-M_5 were from Meru County,M_6-M_{11} were from Embu county).

Taxonomic treatment of *Catha edulis*

Although the two clusters segregated geographically and there is a possibility of environmental modification or differences in maintenance practices between the two counties, the present chemotaxonomic study has further emphasized the differences between these clusters although identity of the three compounds present in young stems of all the traditional varieties from Meru county and two compounds in young stems of all the traditional varieties collected in Embu county is yet to be confirmed. It is therefore recommended that the two clusters be treated formally as varieties (pending further research) and scientific names suggested for the two varieties as follows:

a) Variety one: consists of all traditional varieties from Meru County hence the scientific name suggested is *Catha edulis* var.*edulis* because it is the first variety most widely known and should therefore take the name of the specific epithet according to the rules of International Code of Nomenclature for Algae, Fungi and Plants (ICN).

b) Variety two: consists of all the traditional from Embu county hence the scientific name suggested for this variety is *Catha edulis* var.*mbeereensis* (Kinyua)named after Mbeere region of Embu county were the variety claims its origin and also occurs extensively in this region. The following field guide to identification of the infraspecific taxa of *C.edulis* found in Meru and Embu counties based on the present study is proposed:

Table 4. 5: Key to varieties of *Catha edulis*

1 (a) Plant a tree…………………………………………………………………2a

1 (b) Plant a shrub…………………………………………………………....2b

2 (a)Branches pendulous, old branches white in colour, branchlets thin and round,

leaves opposite on old branches but alternate on young twigs,

Lanceolate/ovate-lanceolate, asymmetrical, leaf pairs10-20, nodes swollen,

petals white, ovary narrowly

ovoid …………………………………………………………...*Catha edulis* var. *edulis*

2(b) Branches erect, old branches grey in colour, branchlets thick and flattened,

leaves opposite rarely alternate, symmetrical, oblong-elliptic,

leaf pairs 4-8, petals pale white………………..*Catha edulis* var.*mbeereensis*(Kinyua)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Ethnobotany

Since time immemorial, man has been naturally classifying plants and animals. His continued existence has been pegged on his ability to discern similarities and differences between objects and events in physical world and to make known these similarities and differences linguistically. This ability to perceive discontinuities in nature has received a great deal of interest in recent years. The study of folk taxonomic systems is crucial in interpreting the logical processes going on in peoples' minds as well as understanding the application of taxonomic systems themselves (Raven *et al*., 1971).

In the present study, ethnobotanical survey was conducted to determine traditional knowledge of Ameru and Aembu communities of Kenya with special focus on traditional classification and ethnomedicinal uses of *C.edulis*. The approach of collecting folk biological knowledge on *C.edulis* involved mainly communicating with people through face to face interaction. The forty two key informants interviewed comprised more males (32) than females (10) (Appendix II). This is due to the fact that traditionally, the value of *C. edulis* (chewing habit) has been associated with men (Al Motarreb *et al*., 2002b), compared to women in these counties. Thus, cultivation of this crop has been carried out by men for centuries hence they harbor a vast knowledge of the plant than their female counterparts.

The Ameru people recognize five traditional varieties which include:*Kiraa gikiiru(Asili)*,*kigwe (gikieru)*, *Muchuri*,*kithaara* and *kilantune*. *Muchuri* and *Kithaara (nthaara)*are mainly encountered in Tigania region of the county whereas the other traditional varieties have uniform distribution in the county. *Kiraa gikiiru* also known as *asili*(in Swahili) meaning "original" is a popular traditional variety that is highly reputed in Meru County as it is known to produce softer khat which is sugary in taste(according to informants) and has no known negative physiological effects associated with it. It is the most expensive variety and produces popular market grade known as *miraamiiru* ("black miraa")where a bundle of this known as "kilo" (consisting of about 10 small bundles tied into one bigger bundle) may be sold up to Ksh 4,000.

(Carrier,2007).*Kigwe* also known as *Kiraa gikieru* is the second known traditional variety in Meru as chewing of excess amount of this produces negative physiological effects such as insomnia and formication.*Miraamieru*("white miraa") derived from this traditional variety fetches very little amount of money in the market. In Meru County, a single piece of land may contain all of the traditional varieties of *C.edulis* distinguishable by experienced farmers only. Taste properties and inebriating effects were the major criteria used by farmers to delimit various traditional varieties of *C.edulis*. The problem with such a criterion is that it is very difficult to identify variations in the field. Such a classification can be biased since it is usually based on individual perceptions and may not represent what is actually happening in nature. In the present study, caution was taken to avoid unnecessary duplication of names given to different varieties of *C.edulis* because of different dialects existing in Meru and Embu County. In addition, several names which exist even in literature only refer to grades of harvested twigs but not the whole tree/shrub names. It was made clear to the respondents that only names of tree/shrub types with some noticeable features in the field were needed but not grades from the harvest.

The Aembu/Mbeere community recognizes six major traditional varieties which include *muguka,muti-mutiri, kibwe, mugwathingi, mugumo* and *gitu*.The cultivation of these traditional varieties is more pronounced in Mbeere region than any other part of the Embu County. However, other regions of the county are emerging with intense cultivation of *C.edulis* such as Runyenjes constituency of Embu Sub-County where large tracts of land have been converted into khat plantations. It is important to note that in the market, the harvest from these traditional varieties is known under a single umbrella name "*muguka*" and hence the other names are only known in the rural set-up. Perhaps this is because *muguka* is the most preferred variety in the market as it is characterized by deep splash of red leaves and rapid psychoactive effects after chewing of few leaves.

Although most of the differences mentioned during the survey (Table 4.2) were difficult to assess in the field because they were based on personal experience of the taste properties and stimulating effects, there was a unanimous agreement by the farmers that the whole group of traditional varieties found in Meru differ from those found in Embu/Mbeere region. The traditional classification of the plant also reveals deeper understanding of this species by the people in the two counties.

The use of taxonomically important characters such as leaf shapes by farmers to distinguish traditional varieties is also an indicator of deep knowledge enshrined in the traditional set-up. For instance, *kithaara* in Tigania region of Meru County is mainly identified by its lanceolate leaf shape. Similarly, all the traditional varieties from Embu County are distinguished from those of Meru by their broader leaves apart from their stronger psychoactive effects.

Although *C.edulis* is well known controversially for its amphetamine-like effects, the people of Meru and Embu counties of Kenya have used the plant as a traditional therapeutic measure against common human diseases. A total of 13 diseases were reported to be treated using *C.edulis*. More cases of the medicinal uses were reported in Meru County (62%) compared to Embu County (15%). The rest (23%) were reported in both counties. With more cases of medicinal uses coming from Meru County, it may be suggested that *C.edulis* might have been in cultivation in Meru for a longer period of time than in Embu County. Therefore, there is no doubt that claims of Carrier (2007) that the origin of Kenyan khat is Nyambene hills (Meru County) would hold true in this case. The respondents treated all traditional varieties as one group in each county when assessing the medicinal value of *C.edulis*. No specific traditional variety was claimed to have a specific cure for a given disease in both counties.

Fresh leaves and young stems were the most used parts of the plant. It is probable that the medicinal value of leaves and stems might have been realized in the pursuit of psychostimulating effects of *C.edulis*. Most of the diseases listed in Table 3.3form part of what has been reported before (Margettes, 1967; Kennedy, 1987; Krikorian, 1984; Kokwaro, 2009). However, few of the cases such as use of khat as an antihelminthic has not been reported in the literature and needs further investigation. In summary, from ethnobotanical survey the following can be concluded:

First, the whole group of traditional varieties from Meru (collectively known as *miraa*) is believed to be different from the group of traditional varieties from Embu county (collectively known as *muguka*). This difference is majorly due to large broad leaves and shrubby habit as well as high potency levels of khat from Embu/Mbeere region compared to narrow leaved and tree habit together with lower potency level of khat from Meru region. In fact, the phrases *"miraa yetu*" (our own miraa) and "*miraa yao*" (their own miraa) are popular in Meru and Embu emphasizing the differences in *C.edulis* of the two regions. Therefore, from ethno-studies there are two major groups. The first group consists of all traditional varieties collected in Meru County. The second group consists of all the traditional varieties sampled in Embu County.

Secondly, the higher number of reported cases of ethnomedicinal uses of *C.edulis* in Meru County compared to Embu County alludes to their differences in periods of domestication in these localities. Either *C.edulis* has been in cultivation for a longer period of time in Meru compared to Embu/Mbeere or perhaps the two groups are different in terms of phytochemical constituents responsible for the medicinal value of the species.

5.2 Numerical taxonomy

Numerical taxonomy also known as multivariate morphometrics is a powerful tool for assessing patterns of variations at specific and infraspecific levels. Its main objective is to draw lines between taxa, ascertain differences between different geographical races or to discover the most important characters that differentiate taxa (Marhold, 2011). Morphological characters continue to be most useful in systematics. In the present study, thirtythree characters/character states (vegetative and floral) were used in numerical taxonomy of *C.edulis.* In vegetative propagated plants like *C.edulis*, their source of variation is mainly environmental modification and mutation.

The contribution of environmental conditions in causing variation within a given taxon is a major problem when one is trying to distinguish characters which can provide reliable results in different environments (Karamura, 1998). In such cases, morphological characters become increasingly disappointing because they cannot fully make differences among taxa (Baum, 1981). Nevertheless, Hawkes (1986) stated that clear morphological differences can be seen in all plants when they are analyzed carefully, the only problem is that they become too numerous and too continuous in their expression. Numerical methods offer ways of treating such characters.

Cluster analysis is advantageous over other methods because it does not require the data to be multivariate normal or conform to other statistical models. Several methods of clustering are used depending on the nature of the data. Unweighted Pair Group Method with Arithmetic means also known as Group Average Clustering may be used. The UPGMA gives more insight into the degree of similarity among the OTUs and whether they form groups/clusters and also indicates the level of variation between taxa. Generally, this method provides the best classification results (Sokal and Sneath, 1963; Sokal, 1986).

C.edulis has no documented infraspecific taxa. Although there is existence of a wide range of morphological characteristics, they are mostly continous hence do not allow discrimination of the species at infraspecific level. This justifies the present use of numerical taxonomy because it is a more sensitive technique in delimiting taxa (Rahman and Rahman, 2012).

The phenogram (Fig. 4.2) based on cluster analysis revealed two distinct clusters that segregated geographically. This means that the differences in environments and maintenance practices between Meru and Embu counties caused the traditional varieties of *C.edulis* grown in these areas to cluster according to their locations. The first group comprised of all the traditional varieties collected in Embu County namely: *Kibwe, mugwathingi, gitu,mugumo, muti-mutiri, muguka A, muguka B*)while the second group consisted of all the traditional sampled in Meru County namely: *Kiraa gikiiru A, kiraa gikiiru B, kiraa gikiiru C, kigwe, kithaara, Muchuri*.

The following characters were important for the first cluster:shrubs, branches erect, old branches grey in colour, branchlets thick and flattened, symmetrical leaves, leaf pairs 4-8 and petals pale white in colour. The second cluster has the following diagnostic features: trees, branches pendulous, old branches white in colour, leaves asymmetrical, leaf pairs 10-20, swollen nodes, petals white in colour narrowly ovoid ovary and petals white in colour.The second cluster was further subdivided into two sub clusters. *Kiraa gikiiru A*, *kiraa gikiiru B*, *Kiraa gikiiru C* and *kigwe* falls under the first sub-cluster because of their unique crimson leaves and dark-red young stems. *Kithaara, kilantune Muchuri*, and unknown type fall under the second sub-cluster because of their narrow, pendulous branchlets as well as lanceolate leaves. Although *Kiraa gikiiru A,B* and *C* where collected in different geographical locations, the present cluster analysis has put them close together because of their unique crimson leaves with purplish margins and dark-red young stems.

All the fifteen traditional varieties studied shared many characters such as: having simple leaves, unicostate venation that is pinnately reticulate, triangular stipules with a tuft of hairs, green sepals with ciliolate margins and basally connate, polypetalous, pentamerous, actinomorphic flower symmetry, dichasial cymes, three stigmas, intrastaminal cup-shaped disc,fruit being a capsule and ovate seeds with a white aril.

Differentiation due to geographical isolation of the plant taxa like in the present study can be attributed to biotic, edaphic and phytosocial factors (Nichols, 1962).In Embu County, the traditional varieties of *C.edulis* are mainly found in lowland areas of Mbeere region which are usually dry. Meru County is part of Kenyan highlands and *C.edulis* prefers well drained soils. In such a case, careful consideration has to be taken to avoid misleading delimitation of taxa due to differences in edaphic or phytosocial factors. Further research especially genecology (the study of the gene frequency of a species in relation to its population distribution within a particular environment) of many samples of *C.edulis* will be a promising approach to ascertain the present delimitation of *C.edulis*. Genecology has been studied in different species of plants to resolve problems of geographical isolation of plant taxa (Baker, 1957).

Al-Thobhani *et al*. (2008) made the first genotypic study of 40 genotypes of *C.edulis* found in Yemen and their results indicated clear genetic differentiation among traditional varieties. This further gives evidence of infraspecific variation within the species *C.edulis*. The placing of the fifteen traditional varieties of *C.edulis* into two major groups by numerical taxonomic methods agrees with results of ethno-studies because certain characters such as habit (tree or shrub) which have discriminated the two groups are also similar characters that are used by farmers to separate the two groups.

5.3 Chemotaxonomy

The present TLC analysis was specific for khatamines of *Catha edulis* (Lee, 1995). The study showed that acid-base extraction technique remains a more reliable method of extracting the khatamines of *C. edulis*.CH_2Cl_2/MeOH (9:1) proved to be a better solvent system compared to conventional EtOAc/MeOH/NH_3 (8.5:1.0:0.5) as two compounds were detected after spraying the plate with ninhydrin reagent compared to only one compound when

EtOAc/MeOH/NH_3(8.5:1.0:0.5) was used as a solvent system in analysis of the leaf extracts of *C.edulis.*

The absence of standards such as cathinone hydrochloride and cathine hydrochloride hampered confirmation of the compounds present. Nevertheless, the results of chemotaxonomic study (based on the number of compounds in fresh young stem extracts) were congruent with those of ethno-studies and numerical taxonomy of *C.edulis.* Two compounds were detected in young stems of members of the first cluster (those collected from Embu County) and three compounds (Figure 5.0) in young stems of members of second cluster (those collected in Meru County). This indicates that the two groups are indeed chemically different.

It is possible that the presence of different number of compounds in different parts (leaves and stems/twigs) could be the reason why residents of Meru County have traditionally consumed young stems compared to residents of Embu County who consume leaves. All the leaves analyzed from all the traditional varieties revealed the presence of two compounds spraying the plates with ninhydrin reagent. There is also a possibility that compounds in leaves could be more concentrated than in stems hence the reason why traditional varieties of *C.edulis* from Embu County are believed to be more potent than those of Meru County.

Conclusions

The results of ethno-studies, numerical taxonomy and chemotaxonomy were congruent and they indicated that *C.edulis* is a heterogeneous species. The present ethno-medicinal study has also shown that *C.edulis* is used medicinally apart from its psychostimulant activity.

The study has shown the presence of variation at infraspecific level. The taxonomic relationship among the traditional varieties of *C.edulis* based on numerical taxonomy correlates with results from chemotaxonomy and ethno-studies. Two distinct taxonomic varieties of *C.edulis* have been identified. Variety one, *Catha edulis* var.*edulis,* which consists of all traditional varieties, collected in Meru County while the second variety,*Catha edulis* var.*mbeereensis* Kinyua, comprises of all the traditional varieties collected in Embu County. There is a possibility of environmental modification due to the manner in which the two varieties have segregated geographically.

The presence of three compounds in fresh young stems of *C.edulis* from Meru County and two compounds in fresh young stems Embu County has supported the present morphological delimitation of *C.edulis*. More analytical and pharmacological studies are required to confirm the identity of the compounds in the stems of *C.edulis* and also determine the active principle in the fresh young stems for a thorough classification of the two varieties identified.

The presence of three compounds in fresh young stems of khat from Meru County could also be used to explain why young stems are preferred by chewers of khat from Meru as fresh leaves are discarded. The stems of the traditional varieties of Embu County revealed only two compounds. Nevertheless, *C.edulis* from Embu County whose leaves are chewed is generally known to be more potent although two major compounds were detected in leaves. This suggests that the concentration of the active principle in leaves might be higher than in the young stems or the young stems of khat from Meru could be having another nervous stimulating compound apart from cathinone and cathine. Decomposition rate of the compounds observed is high especially after extraction is carried out. Future research should take appropriate measures to ensure that this decomposition does not interfere with the desired results.

This study has contributed new information to literature on the known traditional varieties of *C.edulis* mainly cultivated in Meru and Embu counties of central Kenya. A comprehensive study covering all collections from other parts of the country and including wild samples would be necessary to make a more thorough classification, and it would be very useful for further studies to use molecular data.

Recommendations

Based on the results of the present study, the following is proposed:

(1) A comprehensive morphological study covering all collections from other parts of the country and range of *Catha* should be carried out to make a more thorough classification.

(2) Advanced qualitative and quantitative techniques such as the use of HPLC should be applied to determine the quality and quantity of compounds in fresh leaves and young stems of *C.edulis* from different localities to help understand more the chemosystematics of the species *C.edulis*. This would also help to identify the compounds responsible for the medicinal properties of the plant and characterize the compounds found in the present study.

(3) Isolation and characterization of the third active principle in young stems and leaves of the Kenyan *C.edulis* should be carried out. In the previous chemical studies cathinone and cathine have been isolated from leaves of *C.edulis*. In the present study, young stems of traditional varieties of *C.edulis* from Meru revealed two compounds which were very close to each other when observed under UV light (254 nm) while those of Embu County revealed only one compound under UV light (254 nm).The active principle in this case is not known.

(4) Antimicrobial and toxicity tests of *C.edulis* should be carried out to confirm the results of the present ethnomedicinal study.

REFERENCES

Adugna, Y. (2009). Phytochemical studies on khat (*Catha edulis*). M.Sc.Thesis, Addis Ababa University,Ethiopia

Al-Dubai, W., Al-Habori, M. and Al-Geiry, A. (2006). Human Khat (*Catha edulis*) chewers have elevated plasma leptin and nonestrefied fatty acids. *Research*, 26:632-636.

Al-Hebshi,N.N and Skaug,N.(2005).Effects of khat chewing on 14 selected periodontal bacteria.*Journal of Oral Microbiology and Immunology*,20(3):141-146.

Al-Motarreb, A., Baker, K., and Broadley, K.J. (2002a). Khat: Pharmacological and medical aspects and its social use in Yemen. *Journal ofPhytotherapy Research*, 16: 403–413.

Al-Motarreb A, AL-Kebsi, M, and Al-Adhi, B. (2002b). Khat chewing and acute myocardial infarction. *Heart*, 87:279-280.

Al-Thobhani, M.A.H., Sathyanarayana, B.N., Simon, L.and Sondur, S.N. (2008). First comparative genotypic study on khat (Catha edulis Forsk.) genotypes from Yemen. *Middle E. Russian Journal of Plant Science and Biotechnology*, 2: 1–8.

Astalkie, A. Demissie, M., Berhane, Y., and Worku, A. (2015). Oral symptoms significantly higher among long-term khat (Catha edulis) users in Ethiopia. *Journal ofEpidemiology and Health*, 37.http://dx.doi.org/10.4178/epih/e2015009

Ayesha,M.,Zaheerdin,K.,Mushtaq,A.,Muhammad,A.K.,SammiaY.,andHina,M.,(2009).Chemotaxonomic significance of flavonoids in the Solanum nigrum complex. *Journal of Chilean Chemical Society*, 54(4):486-490.

Balint, E. E. (2012). Khat (*Catha edulis):* A controversial plant. Blessing or Curse? Unpublished PhD Thesis, University of Szeged

Baum, B.R.(1981). Taxonomy of the infraspecific variability of cultivated plants.*Kulturpflanz*e 29: 209-239.

Bhargava, V.V., Shashank, C.P., Kruti S.D. (2013). Importance of Terpenoids and Essential oils in chemotaxonomic approach. *International Journal of Herbal Medicine*, 1(2):14-21.

Brenneisen.R and Geisshuler, S. (1985).Psychotropic drugs III: Analytical and chemical aspects of *Catha edulis* Forsk. *Journal of Pharmaceutical Acta Helvetiae*, 60:290-301.

Carrier, Neil C.M (2007). Kenyan Khat: The social life of a stimulant. *Journal of African Social Studies series*, 15:1568-1203.

Cotton, C.M. (1996). Ethnobotany: principles and applications. 1st edition. John Wiley& Sons, Chichester.New York, USA.

Cox and Rampes, H. (2003). Adverse effects of khat:A Review.*Advances in Psychtriac Treatment*,9:456-463.

Crawford, D.J. (2000).Plant macromolecular systematics in the past 50 years: One view.*Taxon*, 49:479-501.

Crockett, S. L.,and Robson, N. K. (2011). Taxonomy and chemotaxonomy of the genus Hypericum. *Journal of Medicinal and aromatic plant science and biotechnology*, 5(1): 1-13.

Crombie,L.(1980).The Cathedulin alkaloids. *United Nations Office on Drugs*,37-49.

Cunningham, A.B. (2001). Applied ethnobotany: people, wild plant use and conservation. 1st Edition. London, UK: Earthscan.

Curto, M.A., Tembrock, L.R.,Puppo, P., Simmons, M.P., and Meimberg, H. (2013).Evaluation of microsatellites of *C.edulis* (qat), Celastraceae) identified using pyrosequencing.*Journal ofBiochemical Systematics and Ecology*, 49:1-9.

Deshmukh, S.A. (2011). Morphometrics of the genus *Cassia* L. from Kolhapur district. *The Bioscan*,6(3):459-462.

Elmi A. (1983). The chemistry of khat in Somalia. *Journal of Ethnopharmacology*, 8:163-176.

Etkin,N.L.(1993).Anthropological methods in ethnopharmacology. *Journal ofEthno-pharmacology*, 38:93–104.

Fadiman A.J. (1994). When we began there were witch men: an oral history from Mount Kenya. University of California press,Los Angeles,

Fluckiger, F.A., and Gerock, J.E. (1887). Contributions to the knowledge of *Catha* leaf.*PharmaceuticalJournal of Transvaal,* 8: 221-232

Fjeilheim, S., Elven, R., and Bronchmann, C. (2001). Molecules and morphology in concert. The*Festuca branchyphylla*complex (poaceae) in Svalbard. *American Journal of Botany*, 88:869-882.

Forsskal P. (1775).Flora Aegyptico-Arabica, sive Descrptiones plantarum,quas per Aegyptum inferioremet Arabiam Felicem Detexit, Ex officinal Molleri, AulaeTypographi, Copenhagen.

Gakuubi, M.M and Wanzala,W(2012). A Survey of Plants and Plant products traditionally used in livestock health management in Buuri district,Meru county, Kenya.*Journal of Ethnobiology and Ethnomedicine.8:39*

Gary J.M. (2004). Ethnobotany: A methods manual. Sterling, London, U.K

Geisshusler, S. and Brenneisen, R. (1987). Phenylpentylamines from *Catha edulis. Journal of Natural Products*, 50:I188.

Gerstle, D.J. (2007).Taming the African Horn's Khat Trade. *SIPA News*, 27–29.

Getahun, A. and Krikorian, A.D (1973). 'Chat: Coffee's Rival from Harar, Ethiopia 1. Botany, Cultivation and use.' *Economic Botany*27: 353-377.

Goldsmith, P. (1988) 'The Production and Marketing of Miraa in Kenya'. In: *Satisfying Africa's food needs* (ed.) R. Cohen, London: Lynne Rienner.

Gonzalez,A.G.,Lopez,I.,Ferro,E.A.,Ravello,A.G,Gutierrez,J.,and Aguilar,M.A.(1986).Taxonomy and chemotaxonomy of some species of Celastraceae. *Journal of Biochemical systematics*, 14(5):479-480.

Greenway, P.J. (1947). Khat. *East African Agricultural Journal,* 13:98-102.

Harborne, J.B. (2002).Phytochemical Methods:A guide to modern techniques of plant analysis.3rdEdition. Chapman&Hall, London.

Hassan, N.A., A.A. Gunaid, A.A. Abdo-Rabbo, Z.Y.Abdel-Kader M.A.K.,and Al-Mansoob (2000).The effect of Qat chewing on blood pressure andheart rate in healthy volunteers. *Tropical Doctor*, 30:107-108.

Hassan, N. A., Gunaid, A. A., and Murray, I. M. (2007). Khat (*Catha edulis*): Health aspect of khat chewing. *Health Journal*,13: 1-13

Hawkes, J.G.(1986). Infraspecific classification-the problems. In: B.T.Styles (Ed.).InfraspecificClassification of Wild and Cultivated plants.Clarendon Press,Oxford.

Hegnauer,R.(1965). Chemotaxonomie der pflanzen (Chemical Taxonomy of plants).Verlag Chemie,GMbH, Gemany.

Islam M.W., Tariq M., Ageel A.M., El-Feraly F.S., El-MeshalI.A. and Ashraf I. (1990).An evaluation of the male reproductivetoxicity of cathinone. *Toxicology*,60:223-234.

Jorgensen, E., and Kaimenyi, J. T. (1990). The status of periodontal health and oral hygiene of Miraa (catha edulis) chewers. *East African Medical Journal*, *67*(8): 585-590.

Kalix P. (1984). The pharmacology of Khat. *Journal of General Pharmacology*15:179-184.

Kalix,P. and Braenden, O.(1985).Pharmacological aspects of chewing of khat leaves. *Journal ofPharmacology*,37:149-164.

Karamura, D.A.(1998).Numerical taxonomic studies of the East African Highlands bananas(Musa-AAA-East Africa) in Uganda.Ph.D Thesis, Department of Agricultural Botany,University of Reading.

Karawya, M.S., Elkieu, M.A, and Ghourab, M.G. (1968). A study of the alkaloids of *Catha edulis* Forsk.growing in Egypt, *Journal of the pharmaceutical sciences of the United Arab Republic*,9:147-157.

Karawya, M.S. and Ghourab, M.G. (1968). Estimation of the Alkaloids of *Catha edulis* Forssk. Growing in Egypt. *Journal of the pharmaceutical Sciences of the United Arab Republic*, 9:159-169.

Karunamoorthi, K., Udeani, G., Babu, S.M., and Mossie, A. (2010). Psychopharmacosocial Aspects of *Catha edulis* Forsk. (Fam.Celastraceae).*African Journal of Pharmaceutical Sciences*, 1(1):112-129.

Khattab NY and Amer G. (1995). Undetected neuro psychophysiological sequelae of khat chewing in standard aviation medical examination. Journal of *Aviation Space and Environmental medicine,* 66:739-744.

Kennedy, J.G. (1987). The Flower of Paradise: The institutionalized use of the drug qat in North Yemen. D. Reidel, Dordrecht, Netherland

KNBS(2015).Website of the Kenya National Bureau of Statistics (KNBS), www.knbs.or.ke, accessed 9/9/2015.

Kokwaro, J.O (1983). An African knowledge of ethnosystematics and its application to traditional medicine with particular reference to the medicinal use of the fungus *Engleromyces goetzei*. *Bothalia*, 14(2):237-243.

Kokwaro, J.O (1993). Medicinal Plants of East Africa. Nairobi: East Africa Literature Bureau.

Kokwaro, J.O. (2009). Medicinal Plants of East Africa. Nairobi. Third Edition. University of Nairobi Press.

Krikorian, A.D. (1984).Qat and its use: An historical Perspective. *Journal of Ethno pharmacology*, 12:115-178.

Krikorian, A.D. (1985). Growth Mode and Leaf arrangement in *Catha edulis* (Kat). *Journal of EconomicBotany*, 39(4): 514-521.

Lawal, O.J, Ayodele AE, and Chukwuka, K.S. (2007). Morphological studies in*Lycopersicon esculentum* Mill. Lines in Southwestern Nigeria. *Journal of Biological Sciences*,7(5):737-744.

Lee,M.M.(1995).Identification of cathinone in khat(Catha edulis.A time study. *Journal ofForensic Science*,49(1):116-121).

Leihmann, T., Geisshusler, S and Brenneisen,R. (1990). Rapid TLC identification test for khat (*Catha edulis*). *Forensic Science International*, 45:47-51

Lemessa,D. (2001).Khat (*Catha edulis*): Botany, distribution, cultivation, usage and economics in Ethiopia. Report UN emergencies unit for Ethiopia.

Mannheimer,C.A.(1999). An overview of chemotaxonomy and its role in creating a phylogenetic classification system. National Botanical Research Institute, Ministry of Agriculture, water and Rural Development, Windhoek.

Margetts, E.L. (1967). Miraa and Myrrh in East Africa. Clinical notes about *Catha edulis.Journal ofEconomic Botany*, 21:358-362.

Marhold,K.(2011).Multivariate morphometrics and its application to monography at specific and infraspecific levels. In book: Monographic plantsystematics: Fundamental assessment of plant biodiversity.Ist edition, chapter 6.A.R.G Gantner Verlag K.G.

Mathys, K.and Brenneisen R. (1993). HPLC and TLC profiles of phenylakylamines of khat (*Catha edulis* Forssk) confiscated in Switzerland. *Journal of Pharmaceutica Acta Helvetiae*. 68:121-128

Mubo, A.S.,Adeniyi A.J.,Adeyemi,E.(2004).A morphometrics analysis of the genus Ficus Linn.(Moraceae).*African Journal of Biotechnology*,3(4):229-235.

Muchena, J.K. (2009). Studies of Capsaicinoids Contents in Locally Grown and Commercial Chillies using Reversed-Phase High Performance Liquid Chromatography. M.Sc. Thesis, East Tennessee State University, U.S.A.

Mulumba, J.W. and Kakudidi, E. (2010). Numerical taxonomic study of *Acacia senegal* (Fabaceae) in the cattle corridor of Uganda. *South African Journal of Botany*,76: 272-278.

Mwenda, J.M., Arimi, M.M., Kyama, M.C. and Langat, D.K. (2003). Effects of khat (*Catha edulis*) consumption on reproductive functions: a review. *East African Medical Journal*,80: 318-323.

National Commission for Science,Technology and Innovation (2006). Report on Kenyan *Catha edulis (*miraa).

Nencini, P., Ahmed, A.M. (1982). Naloxone reversible antinociceptive activity of cathinone, the active principal of khat,in the mouse and rat. *Pharmacol. Res.Commun*, 14:759-770.

Nezar, N.A. and Mohammed, S.A. (2010). Experimental gingivitis in male khat (*Catha edulis*) chewers.

Nichols,D(1962).Taxonomy and Geography.Systematic Association,London.

Nigussie, T.,Gobena,T., and Mossie, A.(2013).Association between khat chewing and gastrointestinal disorders:A cross sectional study. *Ethiopian Journal of Health Sciences*, 23(2):123-130

Nyaga, D. (1997). Customs and traditions of the Meru (1stEd.). East African Education Publishers,Ltd,Nairobi,Kenya.

Nyongesa, A.W., Patel, N.B., Onyango, D.W., Odongo, H.O. and Wango, E.O. (2008).Khat (*Catha edulis*) lowers plasma luteinizing hormone (LH) and testosterone secretion, but increases cortisol levels in male rabbits. *Journal ofEthnopharmacology*,116: 245-250.

Nyongesa, A.W.,Onyango,D.W.(2010). Khat (Catha edulis Forssk): A bane or boon to humanity. *Journal of Ethnomedicine*,28:187-209.

Okach, D.O., Nyunja,A.R.O., and Opande,G.(2013).Phytochemical screening of some wild plants from Lamiaceae and their role in traditional medicine in Uriri District-Kenya.*International Journal of Herbal Medicine*,1 (5): 135-143.

Owuor B.O. (1999).Ethnobotanical and phytochemical study of herbal remedies of Migori District, Kenya.Msc.Thesis, University of Nairobi.

Paris, M.R and Moyse, H. (1958). Abyssinian Tea/*Catha edulis* Forsk., Celastraceae, *Bulletin on Narcotics*,10:29-34.

Pantelis, C., Hindler, C. G. and Taylor, J. C. (1989) Use andabuse of khat (*Catha edulis*): a reviewof the distribution,pharmacology, side effects and a description of psychosisattributed to khat chewing. *Pharmacological Medicine*, 19:657–668.

Perlez, J. (1992). Guns, Greed and Khat Define a Once-graceful Somali City. The New YorkTimes. http://www.nytimes.com/1992/12/07/world/missionsomalia-reporter-s-notebook-guns-greed-khat-define-once-graceful-somali.html.

Pinheiro, F. and de Barros, F. (2007). Morphometrics analysis of *Epidendrum secundum* (Orchidaceae) in Southeastern Brazil. *Nordic Journal of Botany*,25: 129-136.

Rahman,A.H.M.M.(2009).Taxonomic studies on the family Asteraceae(Compositae) of the Rajshahi division.Ph.D Thesis,Department of Botany,University of Rajshahi,Bangladesh.

Rahman,A.H.M.M.(2013).An ethnobotanical investigation of Asteraceae family at Rajshahi Bangladesh.*Academia Journal of Medicinal Plants*, 5(1):92-100.

Rahman, M.Z. and Rahman, M.O. (2012). A morphometrics analysis of *Desmodium* Desv. (Fabaceae) in Bangladesh. *Bangladesh Journal of Botany*,41(2): 143-148.

Raven, P.H., Brent, B., and Dennis E.B. (1971). The origin of Taxonomy. *Journal of Science,New series*,174(4015):1210-1213.

Robards,K.,Habbard,R.P.,and Jackson,E.P.(1997).Principles and practice of modern chromatographic methods. Academic press, San Diego NY.

Robson, N.K.B., Halle,N., Matthew,B,and Blakelock,R.(1994).Flora of Tropical East Africa: Celastraceae, Kew herbarium, U.K.

Royal Horticultural Society, (2001). *RHS colour chart*. Royal Horticultural Society, London

Russell, B.(2002). Research methods in anthropology. Qualitative and quantitative methods. 3rd edition California, USA.

Saif-Ali, R.,Al-Qirbi,A.,Al-Geiry,A,and Al-Habori,M.(2003).Effects of *Catha edulis* on plasma glucose and C-peptide in both type 2 diabetics and non-diabetics. *Journal of Ethnopharmacology,* 86:45-49

Salganik, M.J.and Heckathorn, D.D. (2004). Sampling and estimation in hidden populations using respondent-driven sampling. *Journalof Sociological Methodology,* 34(1):193–239.

Schorno,X,and Steinegger, E. (1978). The phenylakylamines of *Catha edulis* Forsk: The absolute configuration of cathinone.MNAR/7/78.

Simmons,M.P.and Hedin,J.P.(1999).Relationship and morphological character change among the genera of Celastraceae sensu lato(including Hippocrateaceae). *Annals of the Missouri Botanical Garden,* 86:723-753.

Sneath, P.H.A.and Sokal, R.R (1973). Numerical taxonomy, San Francisco.W.H. Freeman & Co.

Sokal, R.R. (1986). Phenetic taxonomy. Theory and methods. *Annual Review of*
Ecology and Systematics,17: 423-442.

Sokal, R.R.and Sneath, P.H.A. (1963). Principles of Numerical Taxonomy. W.H.Freeman, London.

Stockman,R. (1912). The pharmacological action of *Catha edulis* and its alkaloids, *The Journal of Pharmacy and Experimental Therapeutics*, 4:251-262.

Stockman,R.(1912).The active principles of *Catha edulis.The pharmaceutical Journal andpharmacist,* 89:676-678

Stuessy, T.F. (1990). Plant Taxonomy. The systematic evaluation of Comparative Data, Columbia University Press, New Yolk.

Szendrei, K. (1980). The chemistry of khat. *Bulletin on Narcotics,* 3:25-35.

United Nations Narcotics Laboratory (1975). Studies on the chemical composition of khat: III. Investigations on the phenylakylamine fraction. *United Nations Narcotics Laboratory. Report,* 75-1262

World Health Organization (2006). Expert Committee on Drug dependence. Critical Review of Khat. Expert Committee Meeting 28-31 March, Geneva.

APPENDICES

APPENDIX 1:Questionnaire used for collecting ethno-taxonomic and ethnomedicinal data of *Catha edulis (miraa/muguka)* in Embu and Meru counties of Kenya.

Informants' details:

Name...

Gender...

Age...

Occupation..

Education...

Location/Residence...

PART (A): General information on Varieties (Botany)

1.0 How many varieties of *iraa/ilaa/muguka* do you know personally? Kindly mention their local names.

2.0 How many varieties exist in this area/farm? Mention their local names.

3.0 How do you distinguish each variety by?

(a)Morphology (outward/physical appearance)

(b) Physiology (based on level of stimulating effect and other associated effects of chewing miraa)

4.0 Kindly provide a brief history of the origin of each variety

5.0 If you were to plant Miraa/muguka today which variety would choose and why?

6.0 How is each variety propagated?

7.0 The harvested miraa/muguka are graded based on their market value. Kindly mention the grades that you are aware of and give the criteria used in grading them.

Which grade is the most preferred and why?

PART (B): SOCIO-ECONOMIC AND CULTURAL IMPORTANCE OF MIRAA/MUGUKA

1.0 Other than being used for commercial purposes and as a psycho stimulant, mention any social and cultural importance of miraa/muguka to the local communities that you are aware of.

2.0 Do *miraa/muguka* have medicinal value?

(a) Yes ( ) (b) No ( )

2.1. If yes state the:

(a) Parts of the plant

(b)Name of the disease(s) treated.

(c) Method of crude drug preparation.

(d) Mode of the drug administration

APPENDIX 2: Description of the categories of key respondents and their general perception on the origin and negative health effects of chewing *Catha edulis*

S/n		Description of the categories of the key respondents	No. of Respondents	Percentage (%)
1		Gender		
	a	Males	32	76
	b	Females	10	24
2		Level of education		
	a	Formal	15	36
	b	Informal	27	64
3		Age category(years)		
	a	45-54	5	11.9
	b	55-64	10	23.8
	c	65-74	15	35.7
	d	75-84	12	28.6
4		Source of ethno knowledge of *Catha edulis*		
	a	Personal experience	20	47.6
	b	Inherited from parents/grandparents	12	28.6
	c	From close relatives and friends	10	23.8
6		Origin of *Catha edulis* in the community		
	a	Indigenous	30	71
	b	Some introduced from other places	10	24
	c	Not known	2	5
7		Negative effects of chewing *Catha edulis*		
	a	Depends on the amount consumed	10	24
	b	Specific varieties have negative effects	22	52
	c	No negative effects- just a mild stimulant.	10	24

APPENDIX 3:Morphological variation among 15 traditional varieties of *C.edulis* based on Squared Euclidean distance.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1:Kiraa gikiiru A	0														
2:Kiraa gikiiru B	5	0													
3:Kiraa gikiiru C	5	6	0												
4:Kigwe	6	7	7	0											
5:Muti-mutiri	16	19	17	16	0										
6:Kithaara	10	9	9	8	14	0									
7:Muguka A	18	17	15	14	14	16	0								
8:Mugumo	22	23	23	20	10	18	10	0							
9:Muguka B	20	17	21	16	16	16	6	8	0						
10:Gitu	22	25	23	20	12	22	14	10	16	0					
11:Kilantune	9	10	11	9	22	10	14	20	16	22	0				
12:Unknown	12	11	9	10	18	10	14	18	16	20	7	0			
13:Muchuri	12	9	9	10	18	4	16	20	16	24	8	10	0		
14:Kibwe	21	24	20	21	9	21	15	9	17	7	21	15	21	0	
15:Mugwathingi	24	25	23	22	10	24	14	8	14	8	24	18	22	3	0