ANTIMICROBIAL ACTIVITY OF INDIGENOUS PLANTS USED BY PASTORAL COMMUNITIES FOR MILK PRESERVATION IN KILOSA DISTRICT, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN NATURAL PRODUCTS TECHNOLOGY AND VALUE ADDITION OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

Milk is an important part of the diet among pastoral communities in Kilosa district, Tanzania. Its production and consumption derives much food security and economic benefits to the rural people in the district. However, Milk has a complex biochemical composition and its high-water activity and nutritional value serves as an excellent medium for growth and multiplication of many kinds of microorganisms. This shortens the shelf-life of milk. In the framework of research to improve the shelf-life and safety of milk, this study was conducted to explore the indigenous knowledge on milk preservation and antimicrobial activity of the commonly used plants in milk preservation. A survey was conducted on the use of herbs in milk in Parakuyo and Mbwade villages in the district, after which leaves, stems and roots of two plant species of *Dalbergia melanoxylon* and *Combretum imberbe* were collected for laboratory analysis. Biological activity using minimum inhibitory concentration (MIC) serial dilution assay against six milk spoilage bacteria and titratable acidity were determined. The common methods for preservation of milk were boiling, fumigation of milk containers using dried stems and roots of selected plant species and fermentation. Ethanol extracts of the plant parts showed antimicrobial activity against *Escherichia coli, Salmonella typhi, Pseudomonas aeriginosa, Staphylococcus aureus, Streptococcus agalactiae* and *Proteus spp.* Stem extract of *D. melanoxylon* showed highest activity with an MIC of 3.125 mg/ml. The difference in biological activity among the extracts was not statistically significant. Furthermore, there was no statistical difference in lactic acid production between milk samples treated with extracts and non-treated fresh milk samples. The results of this study give credence to the traditional use of *Dalbergia melanoxylon* and *Combretum imberbe* and expand our knowledge on the biological activity of their extracts as potential natural preservatives.

DECLARATION

I, **Cleopatra Nawa Kawanga**, do hereby declare to the senate of Sokoine University of Agriculture that, this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____   _____
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(MSc. NPVA candidate)

The declaration above is confirmed by;

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DEDICATION

This work is dedicated to my loving father Mr. R. Nawa Kawanga and in loving memory of my mum Ms. Phyllis M. Sikalundu M.H.S.R.I.P for being my inspiration at all times. And to my brothers and sisters, cousins, nephews and nieces and to my best friend Miss Joan Kasoka for being an all-weather friend who all had to endure my absence and always safeguarding me with their prayers.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviation	Descriptive meaning
AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
cfu/ml	Colony forming unit per milliliter
CI	Confidence interval
DMSO	Dimethyl Sulfoxide
INT	P-Iodonitrotetrazolium
IUCN	International Union Conservation for Natural resources
MIC	Minimum Inhibition Concentration
MUHAS	Muhimbili University of Health and Allied Sciences
NaOH	Sodium Hydroxide
NBS	National Bureau of Statistics
NCCLS	National Committee for Clinical Laboratory Standards
^{o}C	Degrees Celsius
Pa	Pascal
pH	Hydrogen ion concentration
PHCT	Population and Housing Census, Tanzania
TSHZ	Tanzania short-horned Zebu
VPH	Veterinary and Public Health
WIPO	World Intellectual Property Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them desired shelf-life. Since milk is often sold in areas of the world far distant from the production sites, the need for extended safe shelf-life for milk has also expanded (Rassoli, 2007).

Milk has a complex biochemical composition and its high-water activity and nutritional value serves as an excellent medium for growth and multiplication of many kinds of microorganisms when suitable conditions exist (Parekh and Subhash, 2008). Because of the hand milking system pastoralists, it is not easy to avoid contamination of milk with micro-organisms therefore the microbial content of milk is a major feature in determining its quality. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, cow faeces and grass (Khan *et al.,* 2008). Thus, although milk and milk products are a minor constituent in most diets but contaminated milk is responsible for up to 90% of all dairy related diseases of humans (Shirima *et al.,* 2003). The risk of infection by milk-borne zoonotic diseases such as tuberculosis and brucellosis among other diseases is one of the reasons for public health regulations, which discourages the informal milk markets and consumption of raw or unpasteurized milk (Shirima *et al.,* 2003).

Spoilage of milk occurs as the change of flavour, undesirable coagulation of milk proteins, and the increased concentration of free fatty and amino acids. Immediately after hygienic milking, fresh raw milk contains less than 5000 cfu mL^{-1} (Samarzija *et al.,* 2012). Several

microbes were reported to cause milk and dairy products spoilage. Among them include, *Pseudomonas* spp. and *Bacillus* spp which are the most common isolated organisms in raw or heat treated (pasteurized) milk at the time of spoilage. Other bacteria such as *Bacillus cereus Salmonella* spp. *Staphylococcus aureus*, and *E. coli* are known to be a common contaminant of milk and dairy products that produce different enterotoxins responsible for human infections or intoxication (Higginbotham, 2014). Several techiques were known to be used in the proccess of milk preservation. In rural settings particularly in several pastoralist communities, use of medicinal plants is the commonly used technique for different food preservation including milk. Several plants, herbs and spice extracts have been reported to possess antimicrobial activity against a range of bacteria, yeast and molds (Burt, 2004). Charcoal from *Olea europea, Lagenaria siceraria* and *Olea africana* were documented to be used to coat the inside of milk storage gourds to add flavor/aroma and also to protect the milk against spoilage microorganisms. Therefore, it is important to evaluate antimicrobial activity of indigenous plants which are commonly used by pastoral communities for milk preservation.

1.2 Problem Statement and Justification

In Tanzania, the dairy industry is still dominated by informal milk marketing (Kurwijila and Boki, 2003). The major problem facing the pastoralists is the absence of infrastructure to maintain a cold chain, ignorance of food preservation techniques and financial constraints hinder the resource poor actors to access appropriate technology. As a result of the mentioned challenges farmers experience post-harvest losses due to milk spoilage (Orregard, 2013). Most pastoral communities do not practice classic cow milk preservative methods which are heating and cooling to delay milk spoilage. Instead at household and/or village level, milk is consumed raw which may expose them to milk-borne infections (Asmahan, 2010). Concerns about human health risks from the market pathways need to

be addressed in the context of consumer practices, such as use of indigenous plants, to delay or eliminate potential microbes with potential to cause milk-borne infections. Furthermore, despite the important contribution of cow milk to pastoralists, little is known about the postharvest handling, preservation and processing methods in Kilosa district. This study, is therefore, aimed at exploring indigenous knowledge in identifying the existing postharvest handling, preservation and processing practices in Kilosa District, Morogoro Region, Tanzania and testing the antibacterial activity of plant extracts from the plants used in milk by pastoralists. Understanding of these practices will help to design appropriate strategies to enhance the contribution of cow milk to food security for the pastoral communities, especially the poorer households.

1.3 Objectives of the study

1.3.1 Overall objective

To assess indigenous knowledge and antimicrobial activity of traditional herbs used in preservation of milk in Kilosa District, Tanzania.

1.3.2 Specific objectives

i. To explore existing indigenous knowledge, skills and practices associated with use of herbs for milk preservation in Kilosa District.

ii. To establish inventory of plants used to preserve milk among the pastoral communities in Kilosa district.

iii. To determine biological activity of plant extracts against milk spoilage microorganisms using *in-vitro* antimicrobial assay.

iv. To assess preservative effect of selected plant extracts in raw fresh milk.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Milk

Milk is a yellowish-white non-transparent liquid secreted by the mammary glands of all mammals. It is the primary source of nutrition and sole food for offspring of mammals before they are able to eat and digest other types of food. Milk contains a balanced form of all the necessary and digestible elements for building and maintaining the mammalian body (Pandey and Voskuil, 2011). Milk is an excellent source of high quality protein, vitamins, minerals such as calcium and phosphorus. Fresh milk has a pleasant soft and sweet taste and carries hardly any smell. Almost 87% of milk is composed of water and the remaining part comprises total solids (carbohydrates, fat, proteins and minerals) contained in a balanced form and digestible elements. Its composition is not constant, the average percentages of milk components vary with species and breeds of animal, season, feeds, stage of lactation and health and physiological status of a particular animal (Khan *et al.,* 2008).

2.1.1 Safety and quality of raw milk

The safety of raw milk like any other food is of a worldwide concern where various efforts are directed, since it is associated with food-borne diseases, (Ngalasa *et al.,* 2015). Quality milk therefore is milk which is free from pathogenic bacteria and harmful toxic substances such as antimicrobials and other chemical residues like acaricides, free from sediment and extraneous substances. Quality milk should be of good flavor, with normal composition and acidity, adequate in keeping quality and low in bacterial counts. Thus, milk safety and quality is the combination of physical, chemical and microbiological qualities of milk, (Khan *et al*., 2008).

Both acidity and pH of milk are used as indicators of quality (Hossain *et al.*, 2011). Milk acidity is expressed as percentage lactic acid because lactic acid is the principal acid produced by fermentation after milk is drawn from the udder. Fresh milk does not contain an appreciable amount of lactic acid. Therefore, an increase in acidity and decrease of pH is a rough measure of its age and bacterial activity (Hossain *et al.*, 2011). Within a short time after milking, the "acidity increases and pH decreases" due to bacterial activity and release of carbon dioxide (Hossain *et al.*, 2011). The degree of bacterial contamination and the temperature at which the milk is kept are the principle factors which influence acid formation and decrease of pH.

Bacterial contamination of raw milk can originate from different sources: air, milking apparatus, feed, soil, feces and grass. The number and types of micro-organisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health (Karmen and Slavica, 2008). Other bacterial sources are from air, milkers, milk handlers, drugs or chemicals used during treatment of animal and from water used for adulteration by unscrupulous and unfaithful workers/sellers who may be contaminated and may cause additional health problems. Exposure of milk to these sources or conditions may lead to increased microbial contamination and affect its quality (Karimuribo *et al.,* 2005). Therefore, poor bacteriological quality of milk normally results from poor practicing of hygienic principles at the farms, which includes poor handling and transportation of milk. The principles include immediate cooling and storage of raw milk in proper container or equipment (Khan *et al*., 2011).

Undesirable changes in milk are caused by microbial growth and metabolism or by chemical reactions. The determinants of shelf life of fresh milk are usually the spoilage

bacteria that have the ability to grow at different temperatures. This microbial growth induces changes in the taste and odor of milk such as sour, putrid, bitter, malty, fruity, rancid and unclean (Hossain *et al.*, 2011). The common causes of milk spoilage are coliforms; a common form of coliform bacteria is *Escherichia coli* which is used as indicator of the presence of pathogens in water as well as dairy products. Coliforms can cause rapid spoilage in milk because they ferment lactose with the production of acid and gas, and they can also degrade milk proteins (Fadaei, 2014). Others include psychrotrophs which comprise the largest percentage of bacteria in milk and cause spoilage in refrigerator temperatures at or below 7 °C, these are heat stable proteolytic and lipolytic extracellular enzymes producing bacteria which include species of *Pseudomonas fluorescens* and *pseudomonas fragi.* Some species and strains that can survive pasteurization temperature and grow at refrigeration temperatures include *Bacillus, Clostridium, Cornebacterium, Arthrobacter, and Lactobacillus, microbacterium, micrococcus* and streptococcus (Samarzija *et al.,* 2012).

Food poisoning is still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage microorganisms in foods (Jałosińsk and Wilczak, 2009). Consumers are also concerned about the safety of foods containing synthetic preservatives. Therefore, there has been increasing interest in the development of new types of effective antimicrobial compounds. There is growing interest in using natural antibacterial compounds, such as extracts of spices and herbs for food preservation (Jałosińsk and Wilczak, 2009).

2.2 The Uses of Plants in Milk Preservation

The use of traditional plants is of tremendous importance in many societies, including most rural African communities (Bussmann *et al*., 2006). Plants are considered as important resources in providing novel antimicrobial compounds of commercial value. However, studies related to the isolation of bioactive potentials from plant resources against food borne pathogens are limited**.** Since ancient times, plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties (Al-Dhabi *et al.,* 2012).

Several plants were reported to be used to prevent milk spoilage in different parts of the world. Mureithi *et al.* (2000) documented the use of use charcoal from *Olea europea L ssp Africana* (Mill.) to ferment milk to a product famously known as mursik (a local term for fermented milk). This plant was used by the meru people to smoke the inside of storage gourds in order to add flavor and aroma to milk at the same time has effect on bacteria which cause milk spoilage. In Maasai community in Kajiado district, Kenya Raw milk is filled into a traditionally treated gourd made from the hollowed out dried fruit of the plant *Lagenaria siceraria*. The dried calabash, used as fermentation gourd is gently rubbed with a burning end of a chopped stick from the tree *Olea Africana* locally known as Enkidogoe allowing charcoal to break inside. This procedure is repeated at least three times. The gourd is filled with milk and then closed by a special cap obtained from the same gourd during its preparation (Onyango *et al.,* 2014).

2.2.1 *Dalbergia melanoxylon* (Mpingo)

Dalbergia (family Fabaceae) is a large genus of small to medium size trees, shrubs. The species is widespread in tropical Africa, from Senegal and Cote d'Ivoire in the West, to Kenya and Ethiopia in the East, and extending South to South Africa. The hard, heavy

wood is fine grained, resistant to insect attack and is one of the most valuable timbers in Africa. The timber (Mpingo) is widely used in carpentry, construction, musical instruments, ebony carvings, walking sticks, furniture, tool handles and art work. It is mainly the intensive exploitation of its wood, which makes it endangered in Kenya. The foliage and pods are used as forage and fodder for animal and pods are eaten by livestock. The species has various local medicinal uses (bark, roots and leaves) (Sacande *et al.,* 2007).

The bark of *D. melanoxylon* has been used for cleaning wounds while the roots have been used to alleviate abdominal pains, as an anthelmintic and as a part of preparation for the treatment of gonorrhea. The leaves are boiled in soup and drunk to relieve pain in joints (Mutai *et al.,* 2013).

2.2.2 *Combretum imberbe* (Mtagalala)

Combretum imberbe is restricted to the African continent, reaching its northern distribution limit in Tanzania, and southern limit in KwaZulu-Natal in South Africa. Economic value of the tree includes indigenous woodcraft industry, fuel wood, Building materials**,** and feed for livestock and game, (Herrmann *et al*., 2003).

The significance of *C. imberbe* contribution to medicinal uses, both in terms of quantity and importance, is uncertain. Some claims are that the roots are also used to make a decoction that is taken orally to treat diarrhea, while an infusion made from root bark is used for the treatment of bilharziasis (Herrmann *et al*., 2003). Milk preservative uses have not been reported.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted in Kilosa District, Morogoro Region, and east of mainland Tanzania. The district lies between latitude 5 °55´ and 7°53´ to the South of the Equator and longitude 36°30´ and 37°30´ east of Greenwich meridian. According to the 2012 National census, Kilosa District had a population of 438 175 people (218 378 males and 219 797 female) (NBS, 2013).

Parakuyo and Mbwade villages, Kilosa district were selected to be used as study areas because milk is one of the predominant contributors to household income. The area is dominated by the pastoralists of the Maasai tribe. These are nilotes tribe (people) known in East Africa and to the whole world as cattle keepers (Maundu *et al.,* 2001).

Figure 1: Map of Kilosa district Obtained from Google map (sited on 14th July from

http://www.maplandia.com/Tanzania/Morogoro/kilos)

3.1.2 Study design

This was a cross-sectional study carried out in December 2015. A well-structured questionnaire with open and closed ended questions were used to gather information about traditional plants used for milk preservation during survey. Collection of plant part samples was done following information from experienced respondents and the plants were then confirmed by the botanist before collection for laboratory analysis. Several plant parts such as roots, stems and leaves were collected and transported to the faculty for further analysis.

3.1.3 Sample Size Determination

The sample size was determined based on Kothari (2004) formula n= Z^{2}p (1-p) / d^2 for sample size determination for an unknown population. Where, N= desired sample size, Z= standard normal deviation set at 1.96 to 95% confidence interval, p= proportion in the target population estimated to have particular characteristics (prevalence), q=1-p (expected population not having particular characteristics (1- prevalence) and d= degree of accuracy set at 0.05. Assuming that 50% have knowledge on the use of herbs in milk, P= 0.5.

Estimation of sample size was calculated using the following formulae.

$$N= (1.96)^2 \, 0.5(1\text{-}0.5) \div (0.05)^2$$
$$N= (3.84) \, (0.5) \, (0.5) \div (0.0025)$$
$$N= 384.16$$

Due to financial constraint and time only 10% of the calculated sample was adopted

$$0. \quad 361 = 38 \text{ respondents.}$$

Therefore, a total of 38 respondents were involve in the study.

3.1.4 Selection of study villages

Two villages Parakuyo and Mbwade were selected for the survey. Selection of village and household to be involved in the survey was done by purposive sampling with the help of the district veterinary officer (DVO) from the district office. The inclusion criteria was based on the pastoralism and gender whereby at the house hold level only women were selected because they are the ones involved in the milking and milk handling.

3.2 Data collection

A total of 50 questionnaires were administered, 20 from Parakuyo village and 30 from Mbwade village. The uneven distribution of questionnaires between the villages was attributed to the number of households that were accessible during the period of visit.

A structured questionnaire (Appendix) with both closed and open- ended questions with three sections: demographic information, cattle ownership and milk production information and knowledge on the use of herbs in milk.

3.2.1 Administration of questionnaires

The questionnaires were administered by Kilosa District Veterinary Officer (DVO) through face to face interviews to gather data concerning practices in milk storage. The interviews were conducted through the help of an interpreter who interpreted the questions from English to Kiswahili, the national language which the respondents understood. Information including the use of plants, availability and method of use of plant parts, preference of plants for certain use was gathered.

Figure 2: Administration of questionnaire in Kilosa district, Tanzania in December 2015

3.2.2 Direct observation

In qualitative research, direct observation enables the researcher to watch certain things of interest, hence further probing of issues that were not covered in the interviews. The researcher does not try to become a participant in the context; however, he or she strives to

be unobtrusive as possible to avoid bias (Hancock *et al.,* 2006). In this study, direct observation of the fumigation of the milk gourds was done was done.

Figure 3: Maasai girl preparing by cleaning the gourd and followed by fumigation procedure

3.2.3 Ethical consideration

Research permit was obtained from the Vice Chancellor Sokoine University of Agriculture. The purpose of the research was explained to the village leaders after which verbal consents were obtained. At household level, consent was obtained through self-introduction and explanation of the purpose of the study. To ensure confidentiality other than the questionnaire order number, no other respondent particulars like names were obtained.

3.2.4 Data analysis

The questionnaire responses were stored in Microsoft Excel. The data was analyzed for descriptive statistics using IBM SPSS version 20.

3.3 Herbal Plant Sample Collection and Identification

3.3.1 Plant sample collection

Based on the information from questionnaire interviews, three main plants were mentioned to be used among the Maasai women of Parakuyo and Mbwade village of Kilosa district. The trees in Kiswahili were Mpingo, Mtagalala and Mtego. However, the Mtego plant was not available within the villages; it was being bought in its dry usable form from traders who obtained it from a distant place. Therefore, only two species (Mpingo and Mtagalala) of the plants were collected for Laboratory analysis in this study. These plants were obtained from Parakuyo village only because it's the nearest Village between the two villages from Sokoine University of Agriculture.

3.3.2 Plant species description

Before collection of plant material for further analysis, the plants were identified and authenticated by a botanist Professor Ruwoichi P.C Temu from Department of Forest Biology, Sokoine University of Agriculture.

3.4 Extraction of Essential Oils

The fresh leaves samples were distilled in a Clavenger apparatus for two hours using distilled water. The process is known as hydrodistilation. Then, the apparatus was rinsed using dichloromethane.

Figure 4: Clavenger apparatus for steam distillation used for extraction of essential oils

3.5 Solvent Extraction of Extract

3.5.1 Plant preparation

Roots, stem and leaves of Blackwood (*Dalbergia melanoxylon*) and Leedwood (*Combretum Imberbe*) were used for examining the antimicrobial activity against six bacterial strains. The plant materials were dried separately under room temperature for three weeks. Then the stems and roots were sliced into smaller pieces. The dry plant materials were ground to powder of approximately 50 microns separately.

3.5.2 Extraction of plant extracts

Two hundred grams of dried ground plant material was added to 1000 mL conical flasks, and 900 mL 96% ethanol solvent was added to each flask. Each mixture was left to extract for 48 hours with hand shaking often to aid the extraction at room temperature. Ethanol was used as the solvent of choice because it is a molecule with both a polar and non-polar end therefore it can extract both polar and non-polar secondary metabolites. Extract was then filtered using a conical flask with side arm, a filter funnel (size 2), and a 90 mm diameter filter paper. Filtered extract was then poured in a round bottom flask. The solvent

was evaporated using a rotary evaporator. Temperature of the water bath was set at 60°C and a pressure of less than 220 Pa. This temperature was used because the evaporation under reduced pressure makes it possible to evaporate at much lower temperatures. Evaporation time ranged from 30 to 40 minutes depending on the type of plant material. After evaporation the extracts were poured in weighed 50 grams empty 100 mL beakers. And the beakers containing extract were weighed again to determine the weight of the sample, the weight of beaker was subtracted from the weight of the beaker and sample.

3.5.3 Preparation of test samples

One hundred grams of each extract was used to make the stock solution; the measured samples were put in 50 g small universal glass bottles. Extracts were then sterilized by filtration using 0.4μm aqua membrane nylon filter disk. 1 ml of dimethyl sulfoxide (DMSO) was used to reconstitute the extracts in order to make a stock solution using volumetric flasks. Working concentration for each sample was 100 mg/ml. The samples were left for 24 hours to allow for proper solubility. Reconstituted extracts were then prepared for the evaluation for antimicrobial properties against the nine bacterial strains. Six samples were coded as R-roots, S-stem and L-leaves. Hence from the two species of plants the samples were labeled according to scientific names and corresponding plant part as **DM-R**, **DM-S, DM-L, CI-R, CI-S and CI-L**.

3.5.4 Sources and maintenance of test organisms

The microorganisms that were obtained and confirmed at the research laboratory of the Department of Medical Microbiology, Muhimbili University of Health and Allied Sciences (MUHAS). They were maintained on Mueller-Hinton agar medium. Twenty-four hour old pure cultures were prepared for use each time. The species of bacteria that were used include, *E. coli* ATCC 25922, *Salmonella typhi* ATCC 27853, *Pseudomonas* ATCC

29953, *Staphylococcus aureus* ATCC 29213, *Streptococcus agalactiae* ATCC 27591 and *Proteus spp* ATCC 13315. The choice of bacteria used was based on the bacteria's potential to be a milk contaminant and cause milk spoilage.

3.5.5 Antimicrobial assay

Suspension of micro-organisms was made in sterile normal saline and adjusted to 0.5 Macfarland standards (10^8 Cfu/ml) (NCCLS, 2000). From the stock of 100 mg/ml extract, serial dilutions were made to 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.39063 mg/ml and minimum concentration of 0.19532 mg/ml. 96 well plates were used for the test. Each sample was put in duplicate. The three controls were DMSO, broth only and standard drug. 30 ml of Mueller Hinton broth was prepared. All 96 plates contained 50 µl of broth. Six columns contained three duplicate samples of 50 µl plant extract, six duplicate columns contained in duplicate DMSO, broth only and standard drug. The initial concentration of each plant extract was 25 mg/ ml. The plates were covered and incubated for 24 hours after which the results were read to determine the effectiveness of the plant extracts against the organisms and the minimum inhibitory concentration of each sample at different concentrations against the bacterial spp.

3.5.6 Minimum inhibitory concentration

3.5.6.1 Microdilution assay

A serial microdilution assay (Eloff, 1998a) was used to determine the minimum inhibitory concentration (MIC) values for plant extracts using tetrazolium violet reduction as an indicator of growth. Color reaction with p-iodonitrotetrazolium violet (Bio Medicals, Inc.) was used in lieu of traditional turbidity measurement to eliminate difficulties of observing turbidity for each of the 96 wells. 100 micro liters of p-iodonitrotetrazolium violet (0.2 mg/mL) in water was added to each well in the 96 well plates and the plates were

incubated for 30 minutes at 37°C. Color changes were observed thereafter. Wells showing purple color indicated the bacterial growth (presence of turbidity) and a well with the lowest concentration of plant extract without any purple color was determined as the minimum inhibitory concentration (MIC).

3.5.7 Statistical data analysis

Data generated was tabulated and discussed in the following chapters. The data was analyzed using Microsoft Excel 2016 by One-way analysis of variance to check for statistical significance difference between the minimum inhibitory concentrations (MIC) of the plant extracts.

3.6 In Vivo Activity of Plant Extracts Against Milk Spoilage Bacteria

3.6.1 Preparation of plant extracts

Different concentrations of each plant extract were dissolved in DMSO from as low as 0.1mg, 0.5mg, 1mg and 10mg being the highest concentration.

Figure 5: Shows dissolved plant extracts in 1ml of DMSO that were used in the study to check the microbial inhibitory activity

3.6.2 Total titratable acidity test

Titratable acidity test is one of the tests that are used to check the quality of raw milk by measuring the milk's chemical characteristics. The Principle behind the test is that a dye, which changes color at a specific pH, is added to the milk, and titrated with a base (added little by little) until the color changes. By recording the volume of base required to bring about color change in the milk sample and the volume of the milk sample, the amount of lactic acid can be calculated. Normal milk acidity ranges from 0.10 to 0.20 % lactic acid. Any value in excess of 0.20 % is attributed to developed lactic acid. Lactic acid development serves as an indicator of the freshness and spoilage of milk. (Draalyer et al, 2009).

3.6.3 Milk treatment

Milk sample obtained from Sokoine University of Agriculture's Magadu farm was used in the study. One hundred milliliters of the milk sample were placed into sample bottles then different concentrations of each plant extract dissolved in DMSO were placed in various milk sample bottles. With milk sample without any addition of plant extract acting as the control.

Figure 6: Milk samples treated with different plant extracts obtained from the test plants

Titratable acidity of the milk samples was determined according to the method of the association of official analytical chemists (AOAC. 1990). Nine milliliters of milk were pipetted into a beaker and 3 to 5 drops of 1% phenolphthalein indicator was added. The milk was then titrated with 0.1 N NaOH solutions until a faint pink color persisted. The titratable acidity, expressed as % lactic acid was calculated using the formula (Gemechu *et al,* 2015).

$$\text{Lactic acid } (\%) = \frac{\text{N/10 NaOH (ml) x0.009}}{\text{Weight of sample}} \times 100$$

3.6.4 Laboratory data management and analysis

The data was presented using descriptive statistics. Statistical significance between the means was analyzed by One-way analysis of variance (One-way ANOVA) using Microsoft Excel 2016.

CHAPTER FOUR

4.0 RESULTS

4.1 Indigenous Knowledge on Post-harvesting Milking Handling Practices

4.1.1 Demographic information

The study included 50 female respondents who owned dairy cows and were willing to participate in the questionnaire interviews. The Fig. 7 below shows the frequency of the age categories. The highest percentages of the respondents were between the ages of 21-30 years old. About 14% of the respondents did not know their ages.
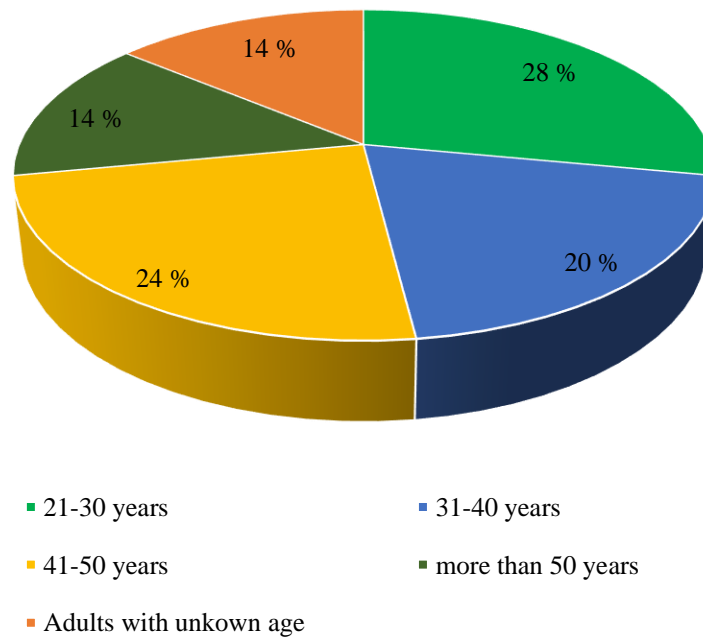
Figure 7: Age categories of respondents from the study on assessment of Indigenous knowledge on the use of herbs in milk in Kilosa district

4.1.2 Types of ownership of cattle

Among interviewed respondents, the majority had cows with less than 20 cows / herd as is presented in Table 1. The majority of the respondents owned less than 20 cows and the main cattle breed in the surveyed areas was the Short-horn Tanzanian Zebu.

Table 1: Cattle ownership, Breeds of cattle owned and Dairy cattle ownership by the women pastoralists in Kilosa district, Tanzania

Number of Cattle	Percentages
below 20	54
21-40	24
40-80	12
above 80	10
Cattle Breeds	
local breed	86
mixed breed	14
Number of Dairy cows	
below 10	50
11-20	36
21-40	10
above 40	4

4.1.3 Milk production

Milk production per day and milk use among the 50 famers incorporated in the study are represented in Table 2 below. With the majority of farmers, they obtain less 5 liters of milk per day. And a highest percentage of the farmers use the milk for home consumption.

Table 2: Milk production; milking times and milk use per day among the 50

pastoralist women

Milking Times	Percentages
Once	18
Twice	82
Milk Production Per Day	
0-5 liters	54
6- 10 liters	24
11- 15 liters	20
above 20 liters	2
Milk Use	
home consumption	54
home consumption and selling	46

4.1.4 Milk markets

For the 46% of respondents that were selling part of their milk, only a few were able to
sale their milk within the neighborhood. The majority (87%) were taking their milk to a
long-distance market area called Kimamba. The results show that there are no milk
collection centers in either of the villages under investigation. This contributed to the high
number of women using the milk for home consumption only. The lack of milk collection
center(s) in the area makes it difficult for the milk producers to find a reliable market.
Milk was sold by a few to a distant market area.

4.1.5 Local practices in post-harvest milk handling

This study shows that six main practices were used by the pastoralist women to handle
milk after milking. The milk was consumed raw by 16% of respondents, it was left to
ferment to sour milk (Mtindi) by others while others chose to treat the milk using plants

and others combined fermentation and milk treatment. Although the Maasai pastoral communities are associated with consumption of raw milk (Kanyeka, 2014), from the results of this study, it shows that only a few are still practicing the consumption of raw milk. The aggregate majority are practicing other methods such as fermentation of the milk, others boil the milk before consumption, and others treat the milk by fumigation with selected plant species.

Table 3: Post-harvesting milk handling practices

Non-treated and non-fermented milk	**16**
Fermentation	8
Herbal milk treatment	12
Fermentation & Herbal milk treatment	26
Boiling & Herbal milk treatment	10
Boiling & Fermentation	14
Boiling, Fermentation & Herbal milk treatment	14

The Table above indicates that traditional milk treatment is the main practice carried out by the pastoralist women.

According to the table below, of the 74% that use plants, 62% mentioned the use of stem and roots for milk preservation. The commonly mentioned plants were Mpingo (Dalbergia melanoxylon), Mtagalala (Combretum imberbe) and Mtego.

Table 4: Plant species used for milk treatment by the pastoralist women in Kilosa

        district, Tanzania

Plant species	Percentage of users
Mpingo & Mtagalala	16
Mpingo & Mtego	43
Mpingo, Mtego & Mtagalala	41
Plant parts used	
Stem	62
Stem & Roots	38

Majority of the women use all the mentioned species depending on which specie is available in storage or one is easily accessible in the nearby bush. They also mentioned that the plant species differ in their characteristics and thus plant preference varies from one woman to the next.

4.1.6 Reasons for use of herbs in milk

There were three main reasons that were mentioned by the 37 pastoralist women that practiced traditional milk treatment using herbs. The main reasons mentioned by 70 % of the respondent were to adds flavor and extend the shelf-life of milk. Fourteen (14%) of the respondents mentioned that the use of herbs helps reduce the effects of lactose intolerance.

4.1.7 Environmental status of the plant species

Based on the interviews, the plants are mainly collected from the bush in their dry form throughout the year. It was found that the plants were naturally occurring and measures were not taken to domesticate them.

4.1.8 Traditional method of milk treatment by pastoralists

The women collect stems and or roots of the mentioned plant species above, if the plants parts are still fresh, they leave them to dry. Once they are dry they chop them into smaller pieces that can easily enter into the milk container (gourds). A gourd is a plant of the family Cucurbitaceae particularly *Cucurbita* and *Lagenaria* or the fruit of the two genera of Bignoniaceae"calabash tree".

The milk containers were fumigated; this indigenous milk preservation technology is locally referred to as Msisilo. The gourds are fumigated with smoke from burned wood of specific tree species mentioned above. The fumigation was by pushing the smoking chips of the burning stem inside the milk gourd and rubbing it on the inside of the milk container until the smoke died out (about 5 to 10 minutes). The residual charcoal pieces were brushed out with special brush and inverting the gourd upside down and tapping it on the outside. Fresh cow milk to be stored was then put inside. The procedure was repeated one or two times. According to the pastoralists' women, if properly fumigated, fresh cow milk could stay for 24 hours at ambient temperatures.

4.2 Antimicrobial Activity of *Dalbergia melanoxylon* and *Combretum imberbe*
    Extracts

The Fig. 8 below shows the percent of mass that is obtained for different plant parts using the same amount of initial mass of 200 g. The Leaves of both plants showed the highest extract yield percentage. More yield means more particulate or biological material is present in the isolated portion. The final mass of extract obtained is influenced by the polarity of the extracting solvent and length of extraction time.

DM - Dalbergia melanoxylo CL- Combretum imberbe;   R- Roots;
L- Leaves; S- Stem
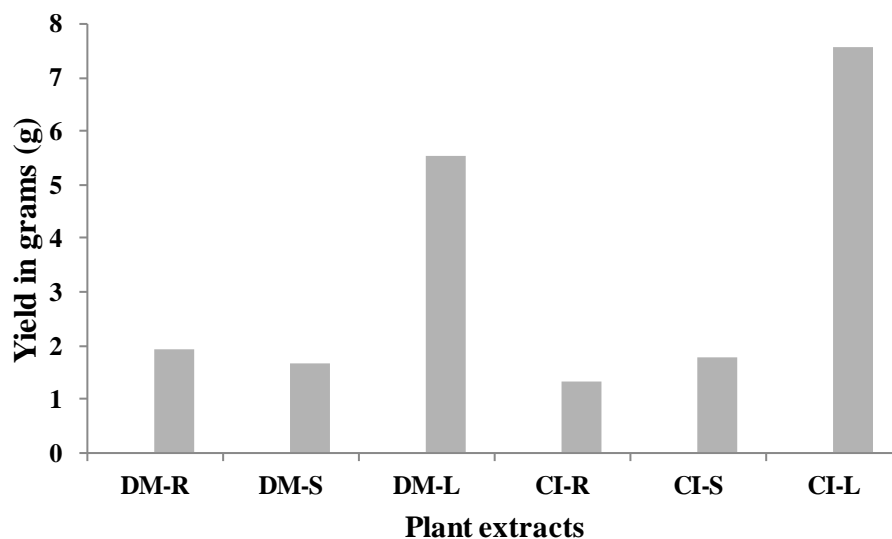
Figure 8: Extract yield following extraction of different plants and parts using

ethanol

4.2.1 Minimum inhibitory concentration (MIC)

The minimum inhibition concentration (MIC) value was defined as the lowest concentration of plant extract inhibiting visible growth of microbes. The highest *in-vitro* activity was exhibited by the stems of Dalbergia melanoxylon with an MIC of 1.24 mg/ml with the least activity been shown by the stems of Combretum with an average MIC activity of 4.17 mg/ml against bacteria.
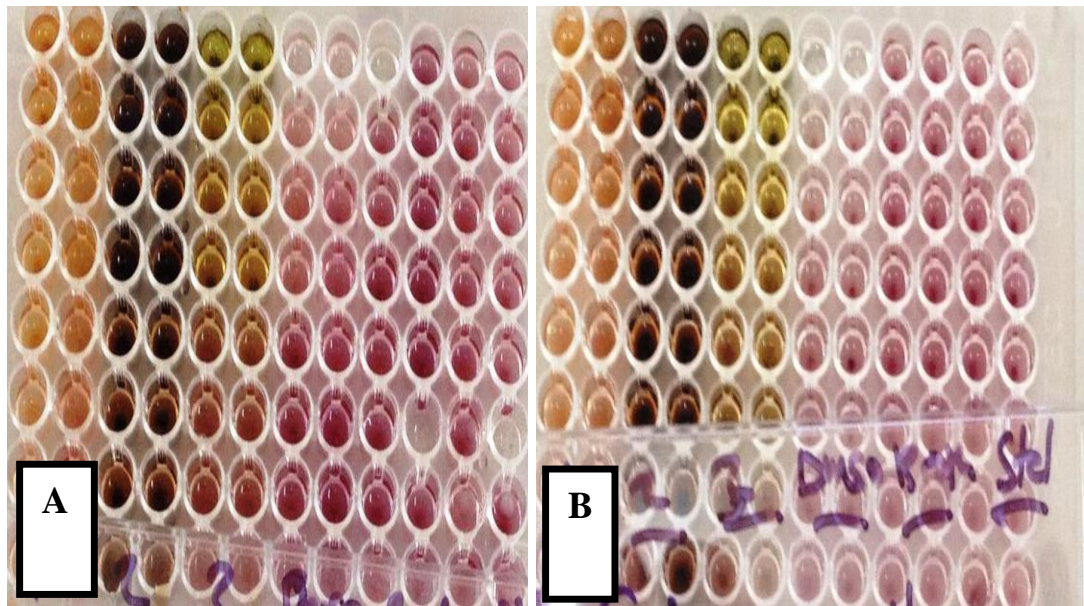
Figure 9: A 96-well plate showing minimum inhibition concentration (MIC) of different concentrations tested for *Salmonella spp* (A) and *Pseudomonas spp* (B)
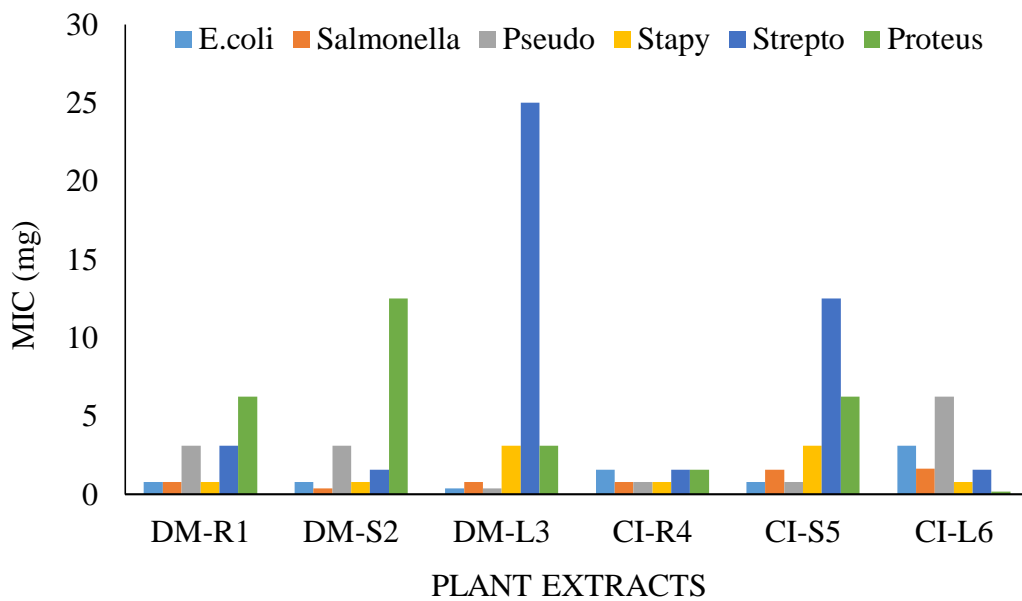
Figure 10: Antimicrobial Activity of the different plant extracts tested against different bacteria spp using ethanol extracts

There was no statistical difference between the antimicrobial activity of the plant extracts against all bacteria and all the bacteria showed no significant difference in their susceptibility to the extracts at (P = 0.67569) even though *Salmonella* was the most susceptible organism.

4.3 Titratable Acidity in Fresh Milk Samples

This study showed continuous increase in titratable acidity in all the samples subjected to 0.5, 1.0 or 10 mg/ml of the plant extracts. After 22 hours, the acidity ranged from 0.29%-0.55% regardless of whether the milk was treated with a specific plant extract or not. The lowest titratable acidity was observed in the leaves of *Dalbergia melanoxylon* leaves although there was no statistical difference between plant extracts (P = 0.999292) or concentrations (P = 0.727105). Hence, the extracts could not prevent milk fermentation.

According to the figure below, from the graphs the increase in titratable acidity seems to have been influenced more by the prevailing ambient temperature.
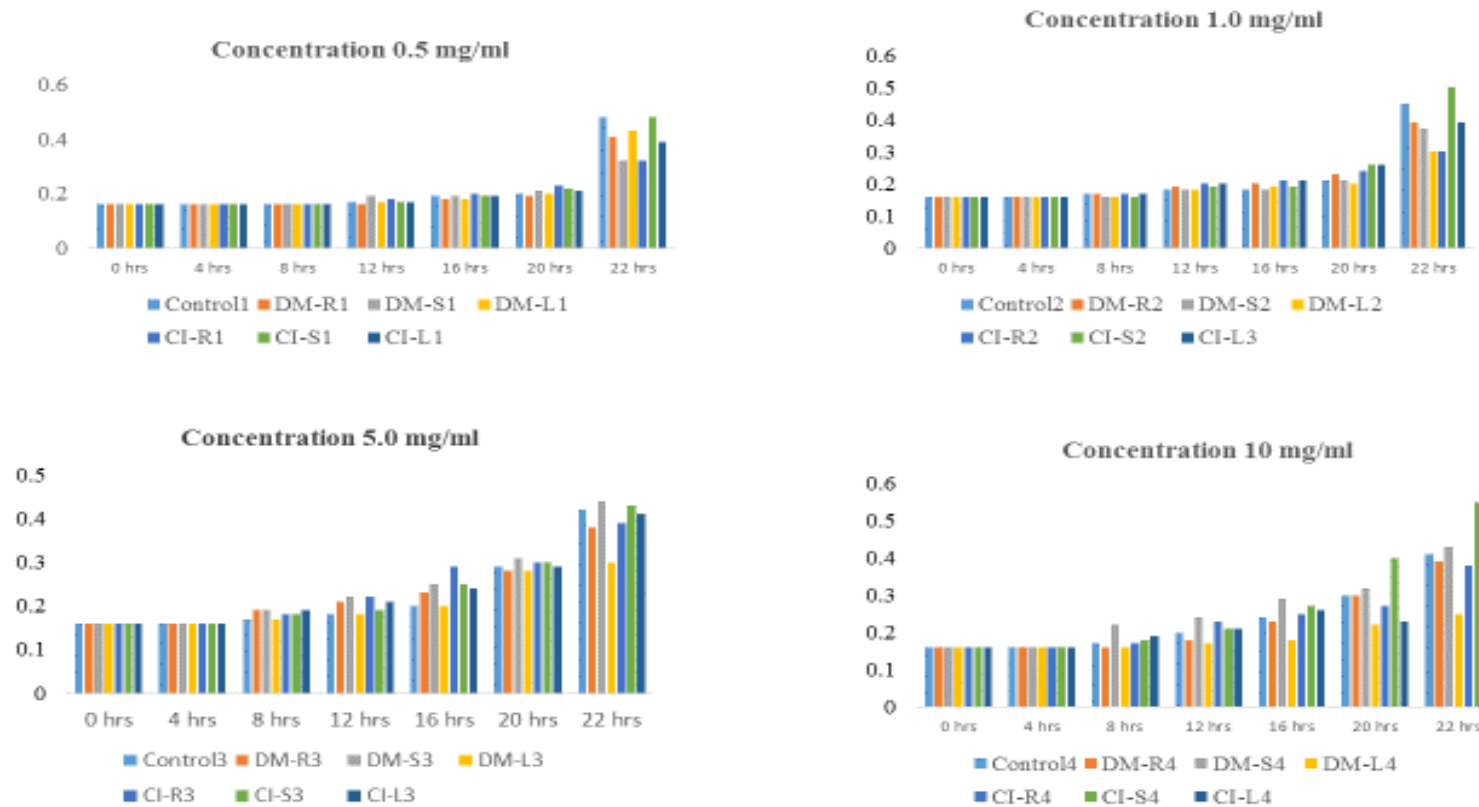
Figure 11: Titratable acidity development as observed in raw fresh milk treated with plant extracts obtained from the plants obtained from Kilosa district, Tanzania

CHAPTER FIVE

5.0 DISCUSSION

The current study assessed indigenous knowledge among the Maasai women in Kilosa district with regard to use of herbs in milk preservation and also assessed the antimicrobial effect of those plants against selected bacteria which cause milk contamination or spoilage. This was done following the fact that the milk market in the area is highly fragmented because the area is situated far from the urban area where there are milk centers. The absence of proper ways to maintain cold chain together with unhygienic milking and handling practices, result in poor milk quality and spoilage. Furthermore, milk is consumed raw by the majority of the pastoralists which may expose them to milk borne diseases such as bovine tuberculosis and brucellosis.

Generally, findings from this study showed existence of indigenous knowledge with regards to the use of plants in milk. As opposed to the long known notion that the pastoralists drink raw milk (Kanyeka, 2014). The study established existence of indigenous knowledge; the knowledge is being preserved as it is being passed on from one generation to the other. Although the women that participated in the study were of varying ages with the highest percentage being less than 30 years of age and only 14 percent were above 50 years of age, all these women were found to possess similar knowledge. This knowledge qualifies to be called indigenous knowledge as it was found to be practiced among a specific community or group of people, women pastoralists.

In this study, it was found that lower proportion of respondents prefer consumption of raw milk rather than boiled milk. It was also observed that women shared similar knowledge with regards to post-harvest milk handling and thus uses the same method of milk

treatment by fumigation of the milk containers. From this study, only three plants were observed to be commonly used in Mbwade and Parakuyo villages. These plants were found to contain antimicrobial activity against potential milk borne bacteria. The observed antimicrobial activity of the plant extracts is an indication of presence of secondary metabolites. The compounds in *Combretum imberbe* were reported by Angeh *et al.* (2007). Likewise, the occurrence of isoflavones, isoflavanones, neoflavones, sterols, anthraquinones, cinnamyl esters and triterpenes in *Dalbergia melanoxylon* has been reported by Mutai *et al.* (2013). However, these plants had no antimicrobial effect in-vivo in fresh milk. Results obtained *in vitro* cannot usually be transposed as to predict the reaction in vivo because the timing and intensity of effect on a given target depends on the concentration time cause of the extract at the site. Significant loss in antimicrobial activity in vivo maybe attributed to the binding effect of the antimicrobial compounds in the extracts to the proteins in milk. The binding effectively reduced the concentration of the free flavonoids available for antimicrobial activity. For a given polyphenolic structure, the ability to bind and precipitate different proteins may vary considerably. Likewise, a protein molecule may show varying order of affinity for different polyphenols (Ansari *et al.,* 2015). Thus, the loss in activity of the plant extracts in vivo in milk is attributed to the binding of the phytochemicals in the extracts to the milk protein casein.

With regards to cattle ownership, all the women reported to own local breed of cattle with less than 20 cattle per herd. In the findings women reported to milk their cows twice each day (i.e. in the morning and in the evening) and in between the cows were allowed to graze as they practice free range grazing Since the breed of cattle is the low milk producing one, the women are only able to obtain less than 10 liters of milk each day depending on the number of lactating cows and the season of the year. During the rain the women are able to obtain more milk because there is more green pasture and thus the cows

are able to eat enough beyond the energy requirement for maintenance thus the extra energy they obtain is channeled towards milk production. A high percentage of this milk produced is used on-farm for household food security as milk especially fermented is a major part of the Maasai diet (Maundu *et al.,* 2001).

For the milk markets, the study established that there is lack of milk markets, thus most of the milk is consumed at household level or sold to neighbors within the village. There are no milk collection centers in these villages for equipped cooling system. Those that are able to transport their milk take it to a distant area called Kimamba where they are able to find customers. Respondents reported that the only milk processing done at village level is letting the milk ferment into sour milk locally called *mtindi.* The results show that the number of those practicing consumption of raw milk has reduced, nevertheless, raw milk is still the most consumed compared to fermented and boiled milk. According to Kurwijila and Boki (2003), boiled milk drank as hot drink is much safer than when it is taken as chilled drinking milk.

The main preservation methods for fresh milk included fumigation of milk containers and boiling of milk. However, some women were found to practice a combination of two or three of these methods of milk preparation prior to consumption. The majority of women combined milk fermentation with traditional milk treatment using plants followed by those who practiced consumption of raw milk and then those who combined three methods. The use of plants to treat milk was common in this community. These results were in agreement to two studies in Kenya and one done in Ethiopia. Though these other studies pointed out that the treatment was done for the purpose of processing and preservation of fermented milk and not to slow down the process of milk fermentation as is the case with women in Kilosa district. According to Shahan and Chandan (1979), fermented milk is of

great significance because it preserves and supplies energy, high quality protein, vitamins and minerals, which enrich the diet. Cultured dairy products have been reported to possess therapeutic value and may play a role in alleviating the problem of lactose intolerance (Vonk *et al*., 2012). In many cultures, fermented foods are of high social value with certain beliefs linked to the consumption of the product. Under given circumstances traditional fermented milks may also serve as a source of income. Milk treatment is generally a preserve of women, women instead of men are involved with milking cows and post milking milk treatment (Mureithi *et al.,* 2000).

Though the women mentioned various plants, there were only three plant species commonly used by the women in these villages. The plant species were identified to be *Dalbergia melanoxylon* and *Combretum imberbe*. The Swahili name of the third plant being Mtego but its scientific name could not be studied because the plant was not available within the boundaries of the two villages. The plant parts that were found to be of importance for the milk treatment were mainly the stems of the mentioned plants. The dried stem cuttings of these plants were used in the fumigation of the insides of the milk gourds used for milk storage. The main reasons that were established for this practice were the first being that the plants impart a certain aroma or flavor to the milk enhancing its palatability, secondly, when the gourds are smoked, it helps prolong the shelf-life of the milk for at least 24 hours. Thirdly, others mentioned that even though they are unable to drink milk due to lactose intolerance, when the milk was treated in this way, it enabled them to drink the milk without experiencing stomach problems as they claimed that the plant species have medicinal value. However, these plants were not combined when used. The women used one plant species only at a particular time. The most preferred was *Dalbergia melanoxylon* for both flavor and preservation of milk freshness. The smoke sterilizes the gourd indirectly preserving the milk. The smoke lines the inside of the gourd,

reducing its porosity rendering it airtight. The smoke from the embers, also has a preservative effect, which prevents undesirable bacterial multiplication that causes spoilage while allowing natural souring.

The procedure of milk treatment (*msisilo*) is similar to those reported in studies done in Kenya. According to Mureithi (2000), milk treatment technology originates from the Kalenjin Community of Kenya for whom milk is a staple diet. The community developed the unique milk preservation technology using indigenous tree species about 300 years ago. The technology evolved as a result of the need to avoid wastage by preserving and storing excess milk for use during the dry season. *Cassia didymobotrya* native to Eastern Central Africa is the most preferred tree species for milk treatment among the Kalejin community. Other tree species used for fermented milk (Mursik) are *Lantana kitu* (Muokiot), *Olea afrikana* (Emitiot) *Rhus natalensis* (Natal Rhus), *Olea capensis*, *Acacia meansii* and *Prunus africana*' among others. According to Wayua *et al*. (2012) in Isiolo District, Northern Kenya, milk processing was the preserve of women, with fresh camel milk and spontaneously fermented camel milk (*suusa*) being the main products. Fresh milk is preserved in a similar manner by smoking of milk containers and boiling. Smoking was the predominant practice, and was for extending the shelf life and also imparting a distinct smoky flavor to milk. The milk containers were fumigated with smoke from burned wood of *Olea africana*, *Acacia nilotica, Balanities aegyptica* and *Combretum* spp tree species. Similarly three indigenous plants namely *Lippia javanica,* Olkingiri and *Olea europaea* used by the Maasai community in Kajiado district to process and preserve milk. This shows that the practice is known among the pastoral communities in different parts of Eastern Africa (Onyango *et al*., 2014).

In order to assess the conservation status of plant species in order to improve and measure conservation efforts, the environmental sustainability of these plants was also evaluated, the results obtained from this study show that these plants are available in the two villages. Women collect them within the villages in their fresh or dried form and sometimes the stem cuttings are bought at the livestock market at low prices such as 1 000 TZS for a small bunch. These plants are fully matured trees that are available throughout the year, therefore, efforts to domesticate the plants by planting them has not been seen as a need. Hence, from these results it can be established that these plant species used for the traditional milk treatment are commonly available within the two villages.

With regard to antimicrobial activity, results in this study revealed that the extracts which were extracted using a polar solvent, ethanol, were active against both gram positive and gram negative bacteria tested that are a common cause of spoilage and contamination in milk. This showed that the extracts of these plants are broad spectrum in their activities. This correlates with the observation of previous workers that plants contain substances that have antimicrobial potential (Negi, 2012). Generally, the plants were more active against the Gram negative compared to Gram positive bacteria. From the results obtained, the most susceptible bacteria to the extracts was *Salmonella typhi* followed by *Escherichia coli* and the least susceptible was *Streptococcus agalactiae*. Gram negative bacteria were more susceptible to the plant extract than Gram positive bacteria which contradict the previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Rabe and Van, 1997). It is therefore theorized that Gram–positive bacteria are more susceptible than Gram–negative bacteria due to the differences in their cell wall structure. Gram–negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics. The roots and stems of *D. melanoxylon* showed highest inhibitory effect

compared to the other extracts and the least bioactive extract were the stems of *Combretum imberbe*. However, these differences between all the extracts were not statistically significant, thus all plant extracts exhibited similar activity across bacteria. The antibacterial activity was expressed at varying degrees depending on the bacterial strain and the dosage of the extract. However, the combined effect of these extracts was not studied. These results do not only show the scientific basis for the traditional use of these plants in traditional milk treatment, but also confirms that ethno botanical approach is a valuable approach when investigating antimicrobial properties of plants.

The antimicrobial activity of the plants confirms their traditional use in milk treatment. Results obtained in this study agree with the work of Mutai *et al.* (2013) who studied the antibacterial properties of the ethanol, citric acid, aqueous, dichloromethane and petroleum ether extracts of the bark of *D. melanoxylon* plants, collected from Masvingo, Zimbabwe. Using the hole-plate diffusion method, each extract type was tested against 7 bacteria (*Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella tyhimurium*, *Staphylococcus aureus* and *Yersinia pestis*. The citric acid extract exhibited the strongest antimicrobial activity. The ethanol fraction showed significant antibacterial activity while the dichloromethane and petroleum ether extracts exhibited no activity against bacteria. It was concluded that bark extracts of *D. melanoxylon* are potential antibiotics. The plant has also been reported to contain a dihydrofuran (melanoxin), a neoflavone (mellanein) and a quinone (4-hydroxy-4-methoxydalbergione) compounds responsible for the antibiotic properties (Mutai *et al.,* 2013).

Preliminary work indicated that *Combretum imberbe* is used widely in Africa *inter alia* for treating bacterial infections. The leaves of *Combretum* species are widely used for treating abdominal disorders (e.g. abdominal pains, diarrhea) backache, bilharziasis, chest coughs,

colds, conjunctivitis, dysmenorrhea, earache, fattening babies, fever, headache, hookworm, infertility in women, leprosy, pneumonia, scorpion and snake bite, swelling caused by mumps, syphilis, toothache and general weakness (Angeh *et al*., 2007). Studies done on *Combretum imberbe* indicate that the plant contains several antibacterial compounds. A few studies have been carried out to isolate pentacyclic triterpenes acids and related glycosidic compounds in the leaves of this species. Triterpenoids of *Combretum imberbe* leaves had antibacterial activity on *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* (Angeh *et al*., 2007).

The mode or mechanism of action of the natural antimicrobials i.e, extracts is due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents, and the generation of hydro peroxides from polyphenols (Negi, 2012).

Based on the *in-vitro* antimicrobial activity of the plant extracts *D. melanoxylon* and *C. imberbe*, it was logical to consider in vivo application of the plant extracts in the milk as preservatives because results obtained *in vitro* cannot usually be transposed as to predict the reaction in vivo. Complex food systems contain components such as fats, proteins, and carbohydrates, which may interfere with antimicrobials. Food contamination is still an enormous public health problem, but may be better controlled by the use of natural preservatives. Improvements in the shelf-life of milk has an important economic impact by reducing losses attributed to spoilage and by allowing the products to reach distant and new markets. New trends in food preservation lead to a reduction in the levels of preservatives and/or to the use of "naturally derived" antimicrobials of animal, vegetable, or microbial origin such as plant derived Neem oil, sugar, lemon, citric acid, rosemary extract and animal derived honey and lysozyme.

From the results of this study it was observed that the effects of the plant extracts in fresh milk were not significant. There was continuous increase in the lactic acid development over a period of time and eventually after 20 hours the milk curds were observed. Some treated milk samples exhibited even higher lactic acid percentage than the control though the difference was not statistically significant. Furthermore, increasing the concentration of the extracts showed no added antimicrobial effect however, it resulted in the change of the color of the milk from cream to different colors depending on the color of the extract. These results agree with those obtained by Daramola *et al.* (2012) showing that the fermentation profile of milk can be altered when such milk is supplemented by plant extracts. In their study, the authors used biberry (*Vaccinium myrthillus*) and liquorice (*Glycyrrhiza glabra*) extracts, both plant extracts enhanced fermentation process as shown by increase in lactic acid of the extract added samples in comparison to the sample without plant extract.

The lack of in-vivo antibacterial activity exhibited by the plant extracts could have been attributed to a number of causes one of which could have been the effect of the ambient temperature which speeds up the multiplication of the lactic acid producing bacteria in the milk. The other reason could have been the issue of solubility of the plant extract causing imperfect mixing between the extract and the milk, this in turn could have resulted in reduced effect of the plant extract. Furthermore, the extrapolation of results obtained from in-vitro experiments with laboratory media to food products is not straightforward as foods are complex, multicomponent systems consisting of different interconnecting microenvironments, the presence of protein, carbohydrates and fat in actual food decreases the antimicrobial effect of the herbs (Negi, 2012). Several studies have recorded the influence of food efficacy of natural antimicrobial but none appears to have quantified it or to have explained the mechanism, although suggestions have been made as to the

possible causes. The greater availability of nutrients in foods compared to laboratory media may enable bacteria to repair damaged cells faster (Burt, 2004). Hence, the level of preservatives required for sufficient efficacy may be considerably higher in food products in comparison with laboratory media, which may negatively impact the organoleptic properties of food (Higginbotham *et al*., 2014).

Only one study has been carried out to assess the antimicrobial effect of plant extracts in milk, in this study the milk was fermented, according to Onyango *et al.* (2014). Water extraction of two plant species *Lippia javanica* and *Olea europea* were applied to fermented milk. The results they obtained showed that the control had a higher titratable acidity of 1.08 - 1.87% while the fermented milk treated with *Olea europea* had a titratable acidity ranging from 0.41- 0.98%. Fermented milk treated with plant extracts had better quality properties than fermented milk without any treatment; therefore, justifying the use of the herbs to process and preserve milk by the Maasai community in Kajiado County in Kenya. It was also observed that there was lower bacterial load in treated fresh milk than the untreated milk (Onyango *et al.,* 2014). The level of preservation is influenced by the type of herb used. In the study by Onyango *et al.* (2014) above, the two plants showed different level of effect in controlling the acid producing bacteria with *Olea europea* (Olive) showing better effect than *Lippia javanica*.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. The results obtained in this study showed that in Kilosa district's Mbwade and Parakuyo villages, they used the same technique to impart flavor to the milk and reduce milk degradation. The technology involved smoking of the insides of the milk storage containers using dry plant stem cuttings prior to addition of fresh milk. The main plants that were involved in this process are *Dalbergia melanoxylon* and *Combretum imberbe* species.

ii. The in-vitro antimicrobial assessment of the leaves, stems and root ethanol extracts of these plants exhibited significant antimicrobial properties against six species of selected milk spoilage bacteria. The stems of *D. melanoxylon* exhibited greater activity compared to the other extracts and *Salmonella typhi* was the most susceptible organism across extracts. The antimicrobial properties of these plants may be of great use for the development of both plant antimicrobials and natural food preservatives.

iii. When preservative effects of the extracts were tested in fresh milk, the extracts did not show significant effect in reducing the development of lactic acid than the untreated milk. And increasing the concentration of the plant extracts did not improve the antimicrobial effect in milk.

6.2 Recommendations

Based on the findings of this study, it can be recommended that:

i. Though there is rich ethnobotanical indigenous knowledge among the Maasai pastoral women of Mbwade and Parakuyo villages in Kilosa district, a way must be found to salvage and record this priceless indigenous knowledge with the view of ascertaining its usefulness in the designing and development of a viable and sustainable local milk preservation method, with an ultimate goal of improved pastoral food security and safety.

ii. The fact that a small percentage of the women practice boiling of milk before consumption re-emphasizes the need of sensitizing the women in these villages to heat raw milk before consumption and create awareness on the health dangers of consumption of raw milk.

iii. Whereas this study assessed the activity of the plant extracts, it would be of great scientific benefit if further investigation can be done to assess both the *in-vitro* and in-vivo antimicrobial activity of the smoke produced by the tested plants.

iv. There is need for enacting conservational measures that will ensure the continued existence of these plants so as to tap their potential as antimicrobials and preservatives.

6.3 Study Limitations

- Little prior research studies in Kilosa district on the application of herbs as preservatives in milk for a comprehensive literature review and proper scale of the scope of the study.

6.4 Way Forward

- Longitudinal effect of time. There was not enough time available for further investigation of the topic such that the preservative effect of smoke from the plants in fresh milk was not investigated.

- There were limited resources to investigate the existence of indigenous knowledge on the use of herbs in milk by other communities in the district such as the Agro-pastoralist communities (Sukuma people) and other non-pastoral communities. Thus, these research findings cannot be used to generalize to other communities of people.

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APPENDIX

Appendix 1: A questionnaire to assess indigenous knowledge on the use of herbs to preserve milk in Kilosa district, Tanzania in 2015

This questionnaire aims to find people's knowledge and practices on the use of herbs in milk preservation among the pastoralist communities. Please note that your answer is completely confidential and your name will not be included in any reports of these results. Your individual answer will not be shared with anyone.

1. Questionnaire number: …………………………

2. Date of interview:.…/………/20………..

3. Region: ………………………………

4. District……………………………

5. Village…………………………….

Demographic Information

1. Gender: ☐ Male   ☐ Female

2. Age:

☐
21 – 30 yrs

☐
31 – 40 yrs

☐
41-50 yrs

☐
More than 50

☐
Do not know or prefer not to say

Cattle Information

3. Do you have cattle?

   1)Yes……...   2) No ……

   If yes continue……

   1) How many cattle do you currently own? (Mention the years) …………….

   2) Which breed of cattle do you own?

   1) Local breed

   2) Exotic breed

   3) Mixed breeds

   3) What is the number of your cattle?

   1) Below 10

   2) 11- 20

   3) 21-40

   4) Above 40

   4) How many milk producing cows do you currently own? Mention number(s)

   1) Below 10

   2) 11-20

   3) 21-40

   4) Above 40

   5) How many times do you milk in a time in a day?

   1) Once

2) Twice

3) Three times

6) When do you milk your cows? Circle all relevant answers

1) Morning

2) Afternoon

3) Evening

7) How many liters of milk do you get per day on average? Mention number(s).

8) Do you sale your milk or use if for home consumption

☐ Milk is sold

☐ Milk used for home consumption

☐ Both

9) If sold:

Where do you sale the milk (mention place) …………...

10) How far is the selling point from the homestead? Mention average hours and means of travel ………………

Knowledge On the Use of Herbs

11) If milk is used for home consumption, how do you consume the milk?

☐ Raw

☐ Processed

12) If processed, how do you process it? Mention practice ………….

13) How do you prevent milk from going sour? Mention practice ………….

14) Do you ever add certain plants to your cow milk for any reason?

1)Yes ……   2) No ……

If no discontinue

15) What plants do you use? List the plant mentioned according to priority ……….

16) How do use the plants? Mention technique ……………

17) What plant part do you use for all the mentioned plants

☐ Root

☐ Bark

☐ Stem

☐ Leaves

☐ Flowers

☐ Fruits

☐ Mixed plant parts

☐ Whole plant

20. Where do you get the plants from?

   1) Naturally occurring

   2) Planted

21. For each of the plants named above, what is the purpose of adding it to the milk?

   (list all purposes)

22. For each of the mentioned plant, how do you prepare and process the plant?

23. What harvesting method(s) do you use to collect the plants?

24. What is the status of the plants for each of the mentioned species?

    1) Rare   2) Common   3) Endangered

25. Which season do you normally collect the plants?

    1) Throughout the year

    2) During the rainy season

    3) During the dry season

26. Can the plants that you use be planted?

    1) Yes

    2) No

    3) Some (mention which species)

The end: thank you for your time