THE PRESERVATIVE POTENTIAL OF THE INDIGENOUS PLANTS COMMONLY USED BY THE MAASAI COMMUNITY IN MILK PROCESSING AND PRESERVATION

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2009

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A thesis submitted in partial fulfillment for the Degree of Master of Science in Food Science and Technology in the Jomo Kenyatta University of Agriculture and Technology, Kenya.

2009

DECLARATION

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

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DEDICATION

I dedicate this thesis to my loving mother, Mrs. Mary Wanjiku Gakuya, who has

been a great mum. I love you mum. God bless you!

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

ANOVA - analysis of variance

BHA - butylated hydroxyanisole

cfu - colony forming unit

DPPH - 2, 2'-diphenyl-1-picrylhydracyl

JKUAT - Jomo kenyatta University of Agriculture and Technology

KDDT - Kajiado District Development Trust

LAB - lactic acid bacteria

LC - lethal concentration

n - number of replicates

N - normal

MIC - minimum inhibitory concentration

MRS - de Man Rogosa Sharpe

P - probability

rpm - rotation per minute

SD - standard deviation

TSS - total soluble solids

UV - ultra violet

w/v - weight by volume

ABSTRACT

The study was done because despite the continued use of the indigenous plants by the by the Maasai community in Kajiado district in milk processing and preservation, their actual preservative potential had not been studied. The study had two components: field study and laboratory analyses. The field study was carried out to obtain information about the indigenous plants used in association with milk by the Maasai community in Kajiado district. This was followed by collection and identification of the plants. Three plants used in milk processing and preservation (*Lippia javanica, Olkingiri* and *Olea europaea)* were collected and used in this study.

The chemical and phytochemical composition of the three plants was determined. The plant materials were sequentially extracted to obtain the plants extracts. The plants extracts were analyzed for their antioxidant and antimicrobial activity. The toxicity of the plants was tested to evaluate the safety of these plants for human use. The quality properties, chemical composition and sensory characteristics of fermented milk prepared with water extracts stored at ambient temperatures for 14 days were also determined.

The plants moisture content was 8.84 – 22.80%, pH ranged from 4.9 - 9.9 and ash 3.20 - 6.69%. All the plants analyzed had varied amounts of minerals; calcium

20.3 - 90.0 mg/100g, magnesium 1.8 - 4.3 mg/100g, zinc 10.3 µg/100g - BDL, copper 3.1 – 28.7 µg/100g and iron 0.1 – 0.8 µg/100g. Phytochemicals namely tannins, saponins, flavonoids, alkaloids, steroids, sterols, terpenoids, flavones aglycones and reducing compounds were present in most of the plants tested except saponins absent in O*lkingiri* and flavones aglycones in *Lippia javanica*.

The water extracts exhibited antioxidative activity; the scavenging effect and the reducing power activity of water extracts ranged from 8-84% and 1.2-1.7 respectively. The water extracts had the potential to inhibit the test microorganisms *Lippia javanica* on *E. coli* and O*lkingiri* on *S. aureus* 9 mm and 11.9 mm zone of inhibition respectively. The minimum inhibitory concentration was evaluated and it ranged from 20.03 - 40.03mg/ml. There was significant reduction of microorganisms in milk treated with the water extracts than the fermented milk without any treatment; *Lippia javanica* on *S. aureus* caused 2.98 log reduction and on *C. albicans* it caused 2.99 log reduction at day 3 of fermentation. The lethal concentration LC_{50} of the aqueous plant extracts against brine shrimps ranged from 5.1 – 26.81mg/ml. Fermented milk with water extracts had better quality properties; total plate count was within the cultured milk range $10^6 – 10^9$. The chemical composition of the fermented milk with water extracts; *Lippia javanica* at day 3 of fermentation had the highest fat content 4.8%. Sensory

characteristics of fermented milk treated with plants water extracts were most preferred; *Lippia javanica* 4.05 out of 8 points.

The results obtained in this study, indicated that the indigenous plants possessed both antioxidative and antimicrobial activity. Therefore these plants may be exploited as source of natural food preservatives. It was recommended that further investigations should be carried out on the specific phytochemicals in the plants and the specific lactic acid bacteria responsible for fermentation of the milk preserved with the plants extracts.

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Livestock and their products are an important resource in Kajiado district for both social and economic significance. Cattle are the basis of the Maasai economy, providing food mainly in the form of milk and meat (Keane, 1998). The region is very dry without permanent rivers and is officially designated as semi-arid. This hampers efforts to encourage crop farming as an alternative to cattle rearing. People tend to give up on this system where crop failure is a far too common occurrence (KDDT, 2007). Therefore, livestock-keeping is the main land use in Kajiado district. However, one of the main problems hindering the development of the livestock industry in Kajiado district is lack of sufficient facilities for milk preservation.

The modern methods of processing and preservation of milk are very expensive and inaccessible to the Maasai community in Kajiado district. Milk being high moisture, nutrient dense food, is highly susceptible to spoilage. In addition, high ambient temperatures prevailing in Kajiado district hasten the spoilage process. An alternative method of preservation is thus required in order to make the livestock industry more viable.

Use of the indigenous plant resources available to process and preserve milk may offer this alternative (Maundu, *et al*., 1999; Kokwaro, 1993). The beneficial exploitation of this indigenous technology to improve livestock products can, therefore, contribute towards poverty alleviation and food security (Fall, 2000; Mbogo, 2000; Mutuku, 2001).

1.2 Significance of Indigenous Plants in African communities

Traditional plant use is of tremendous importance in many societies, including most rural African communities (Bussmann *et al.,* 2006). Apart from providing building materials, fodder, weapons and other commodities, plants are especially important as traditional medicines (Sidigia *et al.,* 1990). Medicinal plants are of great importance to the health of individuals and communities (Edeoga *et al*., 2005). Many of the indigenous plants are used as spices and food plants (Okwu, 1999, 2001). Many tribes in Africa have sophisticated plant knowledge (Barrow, 1996). Western influences have led to an accelerating decline of this tradition (Fratkin, 1996). Most knowledge is still transferred entirely orally in many communities. Therefore, research on natural substances has remained essential both in the developed and developing countries (Karumi *et al*., 2004).

1.3 Utilization of Indigenous Plants by the Maasai community

According to a study on utilization of indigenous plants by the Maasai community, plant life is very important to Maasai community (Maundu *et al.*, 2001). They are mainly used as foods, building and construction material, fuel, human and veterinary medicine and fodder. Plants also find important uses in ceremonies and rituals as well as for brushing teeth and chewing to pass time and exercise the jaws.

1.4 Statement of the Problem

Indigenous plants have been used in milk processing and preservation among the Maasai community in Kajiado district from time immemorial. However, the chemical composition, antioxidative and antimicrobial activity of these plants has not been studied despite their continued use by the community. Lack of this vital knowledge about these plants may have contributed to the insignificant exploitation of these plants scientifically and commercially. Therefore, the study of these plants may form a basis of their commercial exploitation.

1.5 Justification

A variety of indigenous plants have been used in preservation of several foods especially milk and meat in the African society over millennia. There is evidence of their effectiveness and acceptability by the society. However, there

is need to establish the scientific basis for their activity and their documentation before they can be exploited commercially.

1.6 Objectives

The main objective of this study was to identify the plants used in association with food especially in milk processing and preservation by the Maasai community in Kajiado district and then screen them for their antioxidative and antimicrobial activity.

The specific objectives were to:-

1.0 collect and identify indigenous plants commonly used by the Maasai community in Kajiado district in association with food especially in milk,

2.0 determine the chemical and phytochemical composition of these indigenous plants,

3.0 determine the antioxidative potential of these indigenous plants, their antimicrobial activity against the common microorganisms associated with milk and their toxicity,

4.0 compare the quality properties, chemical composition and sensory characteristics of fermented milk prepared with the indigenous plants, fermented milk with indigenous plant water extracts and fermented milk processed without any treatment

1.7 Hypothesis

The indigenous plants used by the Maasai community in Kajiado district in milk

processing and preservation possess preservative potential.

CHAPTER 2

LITERATURE REVIEW

2.1 Indigenous plants used by the Maasai community in Kajiado district

According to a research on Ethnobotany of the Loita Maasai (Maundu *et al*., 2001), different plants and plant parts are used for various purposes. The stem and root bark of a number of species are used to prepare a brown tea-like infusion. From some plants, such as *Zanthoxylum usambarense*, the fruits and the leaves are used to prepare this brown tea-like infusion. In the majority of cases, these infusions serve the dual purpose of a drink and medicine, which may either be curative or preventive. A number of species especially in the genus *Ipomoea* (Convolvulaceae) and several genera in *Asclepiadaceae* have edible tubers. All are characterized by a slight sweet taste and a juicy consistency. As a result they are also preferred for their water. The galls of *Acacia drepanolobium* (*eluai*) are formed at the base of a pair of spines. The fresh soft galls are edible.

Soup is the most important use of plants for food in Maasailand. It is a custom to take soup in which plant extracts have been added. This is done to improve the taste also as a preventive measure against diseases and for curative purposes. The moran may however take some species in soup as a drug or stimulant

(Maundu *et al*., 2001). In most cases the root bark is used. Other forms include the root, stem bark or pieces of stem. The most commonly used soup species are *Acacia nilotica* (*olkiloriti*), *Pappea capensis* (*oltimigomi*), *Carissa edulis* (*olamuriaki*) and *Scutia myrtina* (*osananguruti*). *Acacia nilotica* is the most frequently used soup plant. The root or stem bark is boiled in water and the decoction drunk alone or added to soup.

So far, research has identified some 25 plant products used by the Maasai. Among them are latex from the Ficus tree and roots and barks of various plants which are chewed to alleviate thirst. A second plant gum, which may have hypolipidemic (serum cholesterol-lowering) properties, is produced by a species related to the myrrh plant. Myrrh has been valued since biblical times for its medicinal properties (Keane, 1998). Another source of antioxidants is *Acacia nilotica,* whose bark the Maasai use to flavour their meat soups and milk. Some crude acacia extracts seem to have stronger antioxidant properties than either vitamin C or vitamin E (Keane, 1998).

2.2 Major Groups of Bioactive Compounds from Plants

Plants are an indispensable source of chemical compounds. Plant physiologists in collaboration with chemists and biochemists have been able to isolate and characterize a myriad of chemical compounds from plants (Abdulrahman and

Onyeyili, 2001). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). Many phytochemicals are antioxidants, including carotenoids, and flavonoids. Some phytochemicals, such as isothiocyanates in the cabbage family and organo sulfur compounds in garlic, block the carcinogenic action of chemical carcinogens by helping the body dispose of them (http://www.tuberose.com/Antioxidants.html). The crude extracts with phenolics together with other compounds are increasingly of interest in food industry because they retard oxidative degradation of lipids and they improve the quality and nutritional value of the food (Kinsella *et al*., 1993).

Powers, (1964) has found that, in the presence of glucose, micromolar concentrations of these phenolic compounds inhibited bacterial respiration and reproduction whereas in the absence of glucose they were metabolized. Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds which are in the highest oxidation state. The common herbs, tarragon and thyme, contain caffeic acid, which is effective against viruses (Wild, 1994), bacteria (Thomson, 1978), and fungi (Duke, 1985). On the other hand, antioxidants terminate the chain reactions by removing radical intermediates, and inhibit other oxidation reactions by being oxidized

themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols (http://en.wikipedia.org/wiki/Antioxidant).

Flavonoids are a group of polyphenolic compounds, which have the diphenylpropane (C6–C3–C6) skeleton, ubiquitously found in fruits and vegetables (Masafumi *et al*., 2001). The flavonoid family includes flavones, flavonols, flavanones, flavanonols, flavans, flavanols, leucoanthocyanidins, anthocyanidins, aurones, chalcones, and isoflavones. The structural difference in each flavonoid family results from the variation in the number and arrangement of the hydroxyl groups and the extent of glycosylation of these groups (Rice-Evans *et al.*, 1995). Catechins, the most reduced form of the C_3 unit in flavonoid compounds, were noticed some time ago that in teas they exerted antimicrobial activity (Toda *et al*., 1989.) and that they contain a mixture of catechin compounds. These compounds inhibited in vitro *Vibrio cholerae* O1 (Borris, 1996), *Streptococcus mutans* (Tsuchiya *et al*., 1994), *Shigella* (Vijaya *et al*., 1995), and other bacteria and microorganisms (Thomson, 1978). Galangin (3,5,7-trihydroxyflavone), derived from the perennial herb *Helichrysum aureonitens*, seems to be a particularly useful compound, since it has shown activity against a wide range of gram-positive bacteria as well as fungi (Afolayan and Meyer, 1997). It is admitted that a part of the antioxidant capacity of many fruits and berries is derived from flavonoids (Wang *et al*., 1996) and in fact, all

the major polyphenolic constituents of food shows greater efficacy in these systems as antioxidants on a molar basis than antioxidant nutrients vitamin C, vitamin E and β-carotene (Vinson *et al.*, 1995).

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency (Haslam, 1996). They are found in almost every plant part: bark, wood, leaves, fruits, and roots. Scalbert (1991) reviewed the antimicrobial properties of tannins and listed 33 studies which had documented the inhibitory activities of tannins up to that time. According to these studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria. Coumarins are phenolic substances made of fused benzene and α-pyrone rings (O'Kennedy and Thornes, 1997). They are responsible for the characteristic odor of hay. Coumarins were found *in vitro* to inhibit *Candida albicans.* Hydroxycinnamic acids, related to coumarins, seem to be inhibitory to gram-positive bacteria (Fernandez *et al.*, 1996).

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive (Schmidt, 1988). An anthraquinone from *Cassia italica*, a Pakistani tree, which was bacteriostatic for *Bacillus anthracis*, *Corynebacterium pseudodiphthericum*, and *Pseudomonas*

aeruginosa and bactericidal for *Pseudomonas pseudomalliae* has been described by Kazmi *et al*. (1994). Terpenes or terpenoids are active against bacteria (Amaral *et al*., 1998), fungi (Suresh *et al*., 1997). Food scientists have found the terpenoids present in essential oils of plants to be useful in the control of *Listeria monocytogenes* (Aureli *et al*., 1992.). Oil of basil, a commercially available herb, was found to be as effective as 125 ppm chlorine in disinfecting lettuce leaves (Wan *et al*, 1998).

Saponins are glycosides of steroids, steroid alkaloids (steroids with a nitrogen function) or triterpenes found in plants, especially in the plant skins where they form a waxy protective coating. They are also natural antioxidants (http://en.wikipedia.org/wiki/Saponin). Saponins as natural phytomicrobial agents elicit antibacterial, antifungal and antiviral effects. They constitute important defense mechanisms for the host plant and protect against biotic stress factor. These activities can be utilized in natural food preservation against susceptible strains of bacteria and fungi as well as therapeutics for certain chronic diseases (Naidu, 2000). Heterocyclic nitrogen compounds are called alkaloids. Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae, or buttercup family are commonly found to have antimicrobial properties (Omulokoli *et al*., 1997).

Peptides which are inhibitory to microorganisms were first reported by Balls *et al.*, (1942). They are often positively charged and contain disulfide bonds. Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues (Colilla *et al.*, 1990). They are toxic to yeasts and gram-negative and gram-positive bacteria (Fernandes de Caleya *et al.*, 1972). Fabatin, a newly identified 47-residue peptide from fava beans, appears to be structurally related to γ-thionins from grains and inhibits *E. coli*, *P. aeruginosa*, and *Enterococcus hirae* but not *Candida* or *Saccharomyces* (Zhang and Lewis, 1997).

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Many phytochemicals not mentioned above have been found to exert antimicrobial properties. However, there are reports of antimicrobial properties associated with polyamines in particular spermidine (Flayeh and Sulayman, 1987), isothiocyanates (Iwu *et al.*, 1991), thiosulfinates (Tada *et al.*, 1988), and glucosides (Murakami *et al.*, 1993). Estevez-Braun *et al.*, (1994) isolated a C_{17} polyacetylene compound from *Bupleurum salicifolium*, a plant native to the Canary Islands. The compound, 8*S*-heptadeca-2(*Z*),9(*Z*)-diene-4,6-diyne-1,8-diol, was inhibitory to *S. aureus* and *B. subtilis* but not to gram-negative bacteria or yeasts. Papaya (*Carica papaya*) yields a milky sap, often called latex, which is a complex mixture of chemicals. Osato *et al.*, (1993) found the latex to be

bacteriostatic to *B. subtilis*, *Enterobacter cloacae*, *E. coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Proteus vulgaris*.

2.3 Milk consumption in Kajiado district

Meat, milk and blood have been the traditional staple of Maasai diet (Matthews, 2006). Seemingly old dietary habits are changing fast. An international Livestock Centre for Africa (ILCA) study (Nestel, 1989) states: "Today, the staple diet of the Maasai consists of cow's milk and maize-meal." Further studies by the International Livestock Centre for Africa (Bekure *et al.,* 1991) shows a very great change in the diet of the Maasai towards non-livestock products with maize comprising 12 − 39% and sugar 8 − 13%; about on 1L of milk is consumed per person daily.

Notwithstanding this diversification of the Maasai diet, milk remains the dominant staple, making the diet relatively rich in fat and protein. The availability of milk strongly influences the quantity and type of other foods purchased and the nutritional status of the Maasai. When available, milk and butter provides some two-thirds of the daily energy intake. Reporting data from a 24 h diet recall study in July 1982-June 1983 (Nestel,1985), it was noted that, across wealth classes, women and children on Olkarkar and Merueshi consumed

an average of about 1L of milk/active adult male equivalent (AAME) daily, which corresponds very well with "target" and actual milk offtake per person.

2.4 Milk preservation among the Maasai community

Processing of livestock products is limited. Milk is usually fermented immediately rather than consumed fresh (Suttie, 2001). 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 (Mathara, 1999). 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. After fermentation the product is gently shaken before consumption.

2.5 Chemical composition of fermented milk

The composition of fermented milk products depends on that of the initial milks and on the specific metabolism of growing microorganisms. Modern processes affect milk fermentation under predictable, controllable and precise conditions to yield fermented products of high and standardized nutritional value. Because of the great latitude which the legal provision in general allows, the composition of

fermented milk can vary over a wide range (Fluckiger, 1982). The typical chemical composition is given below, in Table 1.

Table 1:   The typical chemical composition of fermented milk*

Dry matter……………………………………..	c.a. 14-18%
Protein…………………………………………	4-6%
Fat…………………………………………...	0.1 -10%
Lactose……………………………………….	2-3%
Lactic acid……………………………...…….	0.6-1.3%
Carbohydrates including added fruits………..….	5-25%
pH…………………………………………...	3.8-4.6
degree of acidity (°SH)……………….………….	40-70

*(Fluckiger, 1982)

CHAPTER 3

MATERIALS AND METHODS

3.1 Research design

This study was cross – sectional, it had two components: a field survey and laboratory analyses. The study site was the Central Division, Kajiado district (shown on page 17); research was carried out in the Sajiloni and Bissil areas. The field survey was done using a semi structured questionnaire (Appendix 1) administered to the four focus groups in Sajiloni village and in Bissil market. Stratified random sampling was done to collect data about the plants used by the Maasai community in these areas.   The main aim of this field survey was to find out the types of indigenous plants used in milk processing and preservation. Completely randomized design was used to collect data from the laboratory analyses.

3.2 Materials

3.2.1 Plants collection

Three indigenous plants (*Lippia javanica*, *Olkingiri* and *Olea europaea*) were collected from the Maasai community in Kajiado district for botanical identification at the Nairobi National Museum of Kenya and chemical and microbial analyses in the Food Science laboratories, at JKUAT.

AREA OF STUDY - KAJIADO DISTRICT
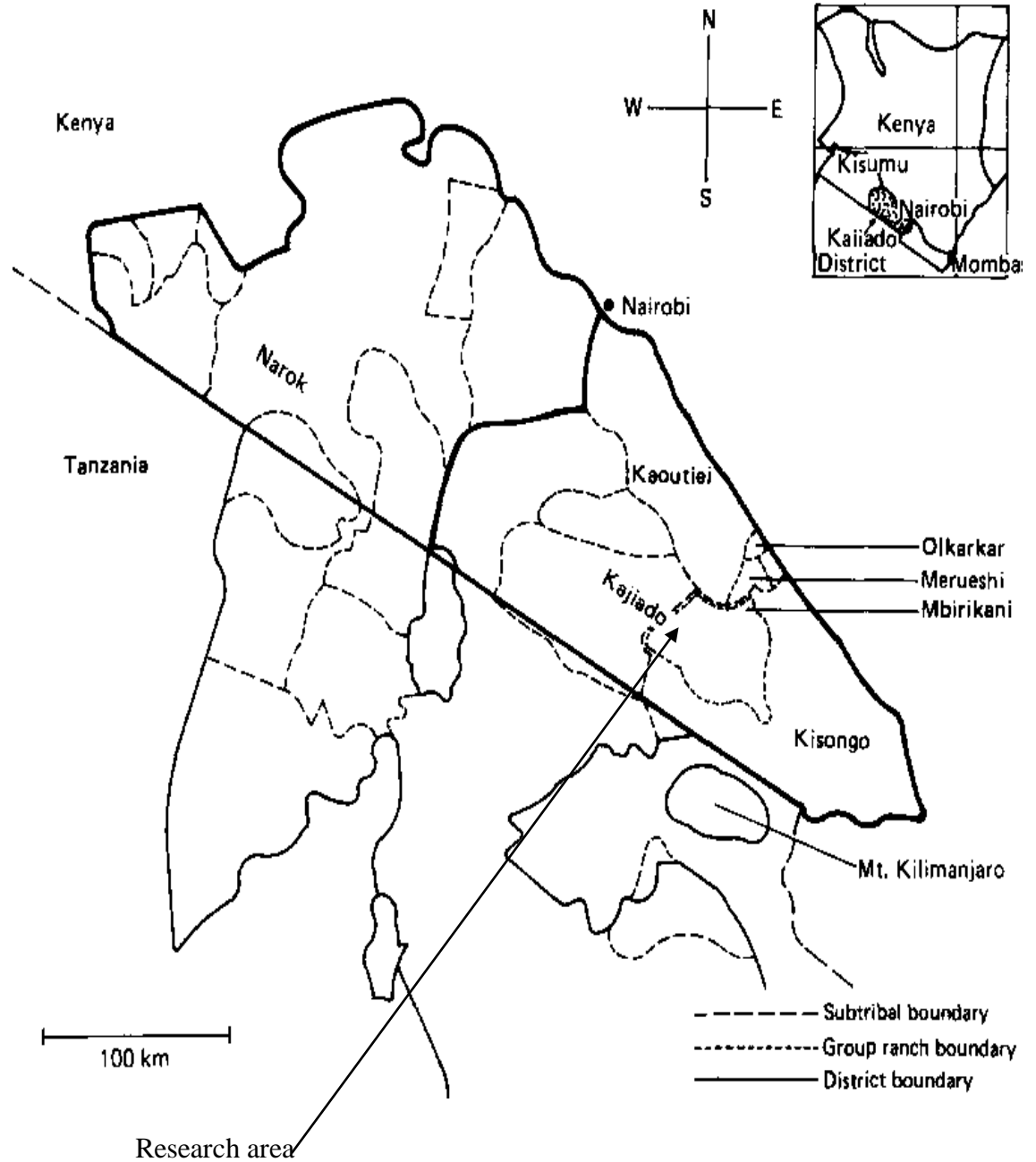
Source: http://www.unu.edu/unupress/food/8F081e/8F081E00.GIF, July, 2007

3.2.2 Preparation of plant extracts

The three plants commonly used in milk processing and preservation (*Lippia javanica, Olkingiri* and *Olea europaea*) collected from the field were chopped into small pieces and then air dried. After air drying they were ashed with fire and then stored in black plastic containers at 5 °C.

The ashed plants samples were sequentially extracted with petroleum ether, methanol and distilled water (1:5) at room temperatures according to Trease and Evans method (Trease and Evans, 1987) with slight modifications. Filtration followed extraction and the filtrates were concentrated *in vacuo*, properly labeled and stored in the refrigerator at 4 °C. The ether, methanol and water extracts were used for qualitative phytochemical determination. Water extracts were used in pH, quantitative determination of phytochemicals and in all microbial analyses.

3.2.3 Preparation of fermented milk samples

Fresh milk was collected from the field, Kajiado district. The fresh milk was pasteurized at 80 °C for 1 min to eliminate background flora. Five batches of fermented milk were prepared from this pasteurized milk. These milk samples were prepared in gourds prepared by the Maasai women from Kajiado district.

One batch was prepared with indigenous Maasai plant (*Olkingiri* stem). In this batch the charred plant stem was applied on the inside of a gourd then the fresh milk was put in the gourd.

Three batches were prepared separately in a gourd with each water extract of the three indigenous plants commonly used in milk processing and preservation by the Maasai community (*Lippia javanica, Olkingiri* and *Olea europaea*) 1:500. The concentration of the water extracts was 40 mg/ml obtained by diluting 40 mg of the water extract in 1 ml distilled water.

Another batch was poured into a gourd without any treatment. The five milk samples were left to ferment naturally at ambient temperature for three days. This is illustrated in Figure 1.

The following parameters were determined after fermentation and storage at ambient temperatures through a period of 14 days; pH, mineral content, protein content, titratable acidity, total soluble solids, fat, ash, changes in microbial quality, survival of microorganisms in fermented milk and sensory evaluation.

Fresh milk

↓

Pasteurization (80 °C 1 min)

↓

Cool to 25 °C

↓

| Charred *Olkingiri* stem | *Lippia javanica* water extract | *Olkingiri* water extract | *Olea europaea* water extract | No treatment |

Fermentation for 3 days

↓

Analyses
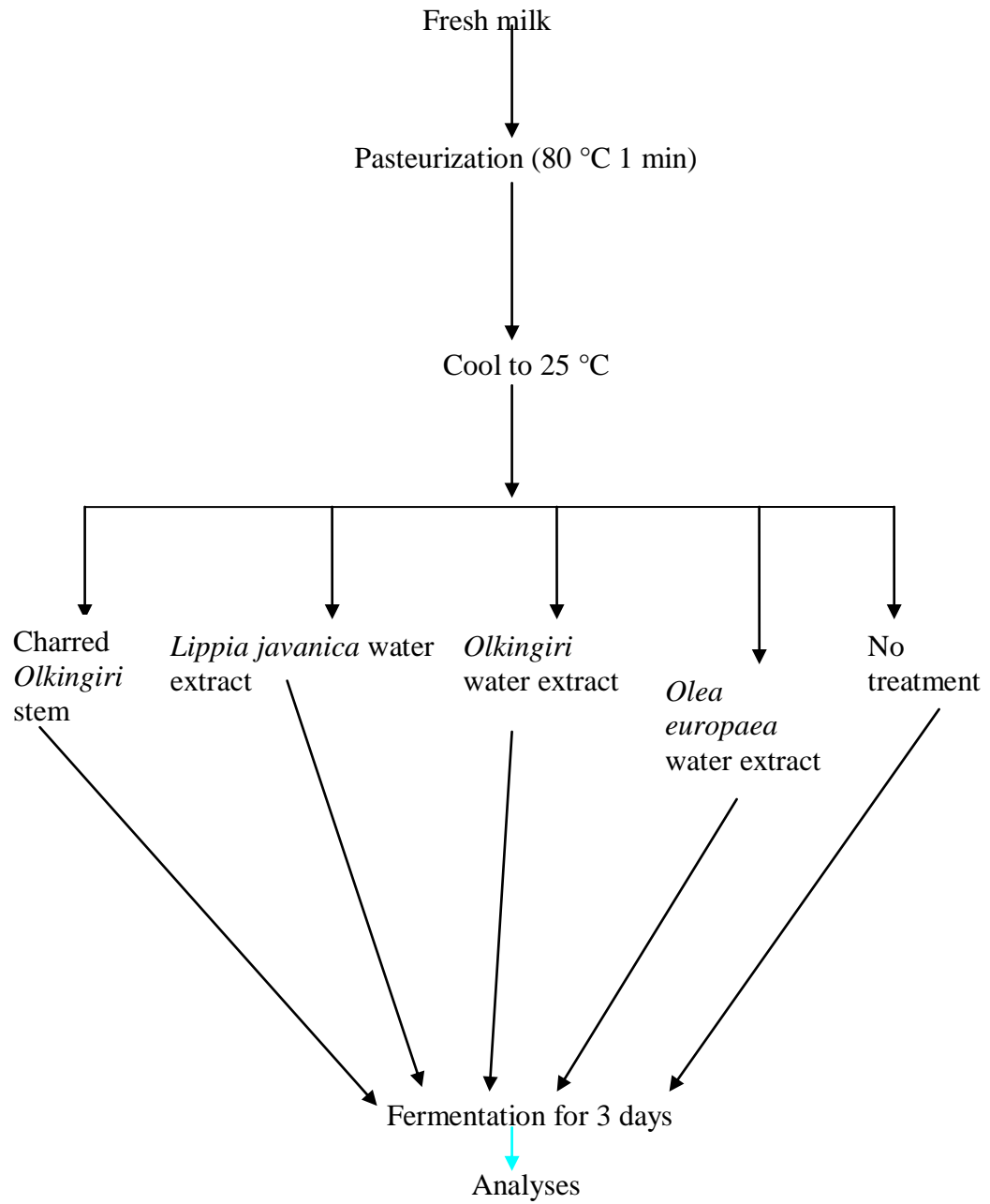
Figure 1:   Flow diagram for preparation of fermented milk samples

3.2.4 Preparation of cultures used in determination of antimicrobial activity of plants water extracts

The following microorganisms of importance in milk industry were used; *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 22923) and *Candida albicans* (ATTC 90028). The pure stock culture of bacteria and fungi was obtained from Department of Botany, JKUAT. The initial concentrations of the cultures obtained were as follows: *E. coli* $3.8x10^9$, *S. aureus* $8.9x10^{12}$ and *C. albicans* $1.6x10^8$. The experimental bacterial strains were prepared by serial dilution of 0.1 ml of bacteria culture into 2.5% (w/v) Nutrient broth No. 2 (OXOID, UK) and incubation at conditions shown in Table 2 to *E. coli* $1.8x10^6$ and *S. aureus* $2.2x10^8$. For yeast 4% (w/v) Malt extract broth (OXOID, UK) was used for serial dilution for *C. albicans* up to $2x10^5$.

3.2.5 Brine shrimp test samples

The brine shrimp (*Artemia salina*) larvae were used in toxicity test. Artificial seawater was prepared by dissolving artificial sea salt (Sigma Chemicals Co. UK) in distilled water. This was filled into incubation tank divided into two unequal compartments separated by a perforated polystyrene wall at room temperature. Brine shrimp eggs (0.5 g) (Kobian (K) Ltd) were sprinkled into the larger compartment, which was darkened by covering with hard paper while the smaller compartment was illuminated with an electrical bulb. The phototropic

nauplii were collected from the illuminated compartment by using a micropipette after 24 h from hatching. These were used for the toxicity test.

3.2.6 Chemical reagents

All the reagents and media used were analytical grade.

3.3 Chemical analyses

3.3.1 Moisture content of the three indigenous plants

The plant materials from the field were air dried for 3 days prior to moisture analysis. Moisture content was determined according to the AOAC method 950.46 (AOAC, 1996) using forced air conventional oven set at 100 °C for 3 h. All determinations were carried out in triplicate.

3.3.2 pH of water extracts the three indigenous plants and fermented milk containing different plants extracts

A small aliquot of 10 ml of each plant water extracts and 10 ml of each fermented milk samples was placed in the measurement cell of a compact pH meter (Horiba B-212) and pH was read out. All determinations were carried out in triplicate.

3.3.3 Ash and mineral content of the three indigenous plants and fermented milk containing different plants extracts

Dry ashing of the plant samples was done according to AOAC method 923.03 (AOAC, 1995) and ashing by gravimetric method 945.46 (AOAC, 1996) was carried out for fermented milk samples. This was done at 550 °C. Mineral analysis was done using Atomic Absorption Spectroscopy (Shimadzu, AA – 6200 Atomic Absorption Flame Emission Spectrophotometer) and the samples were quantified against standard solutions of known concentration ran concurrently. The minerals analyzed were calcium, magnesium, iron, zinc and copper. All determinations were carried out in triplicate.

3.3.4 Protein content of fermented milk containing different plants extracts

The percentage protein content was determined using formol titration method (http://www.wikianswers.com). A sample of 10 ml of fermented milk was pipetted into a 50 ml erlenmeyer flask where 0.4 ml saturated potassium oxalate solution and 0.5 ml phenolphthalein indicator was added. The mixture was set aside for 2 min. The mixture was neutralized by titrating with 0.1 N NaOH to the endpoint which was pink colouration then 2 ml of 40% formaldehyde solution was added and this was allowed to stand for 2 min. Titration was then done again with 0.1 N NaOH to the same endpoint. A blank was run by titrating 2 ml of 40% formaldehyde solution plus 10 ml distilled water with 0.1 N NaOH.

All determinations were carried out in triplicate.

Calculation: % protein= Va - Vb x formol factor

Va = volume of 0.1 N NaOH used to titrate sample after addition of formaldehyde

Vb= volume of 0.1 N NaOH used to titrate blank

Va-Vb= formaldehyde value

Formol factor: 1.74 for cow's milk

3.3.5 Titratable acidity of fermented milk containing different plants extracts

The percentage titratable acidity was determined according to AOAC method 947.05 (AOAC, 1995). To 20 ml of milk sample was added 40 ml of boiled and cooled distilled water, and 2 ml of phenolphthalein (prepared at 1% in 95% ethanol). The mixture was titrated with standardized 0.1 M NaOH until the first color change (to pink) persisted for 30 s. One more drop of 0.1 M NaOH was added and the final volume of 0.1 M NaOH added was noted. The titratable acidity was calculated as follows:

% Titratable acidity = Vg x N x 90 x 100 / Vm

where Vg = volume of NaOH solution added, N = concentration of sodium hydroxide standardized solution expressed in Eq/L, 90 = equivalent weight of lactic acid, and Vm = volume of milk used for titration.

3.3.6 Total solids of fermented milk containing different plants extracts

The standard refractometer method according to AOAC method 925.23 (AOAC, 1995) was used to determine the total solids. The specific gravity of the milk was determined by reading the top of meniscus of Quevenne lactometer. The temperature was observed and the reading L was corrected by 60 °F through the given AOAC standards. The total solids were calculated according to the formula 0.25L+1.2F, in which F = % fat in milk. All determinations were carried out in triplicate.

3.3.7 Fat content of fermented milk containing different plants extracts

The Gerber method (Kirk, 1991) was used in this determination. The temperature of the milk was determined; this was followed by addition of 0.1 ml sulphuric acid into a butyrometer. The sample was thoroughly mixed and 11 ml of the sample was pipetted into the butyrometer. To the mixture 1 ml amylaclohol was added. The rubber stopper was fixed tightly and the butyrometer was wrapped with a cloth and turned upside down a few times while holding the stopper until all the protein is dissolved. The butyrometer was placed upside down in water bath at 65 °C for 10 min until the fat is separated. It was then centrifuged for 5 min at 1200 rpm. It was then put in the water bath for 5 min and the reading was taken. All determinations were carried out in triplicate.

3.3.8 Qualitative phytochemical screening of the three indigenous plants

The plants crude extracts of petroleum ether, methanol and water were subjected to the following qualitative chemical screening for the identification of the various classes of the chemical constituents as described by Sofowara (1993); Trease and Evans (1989) and Harborne (1998).

3.3.8.1 Tannins: A sample of 1 ml of ether, methanol and water extract each was mixed with 10 ml of distilled water and filtered. Three drops of ferric chloride reagent were added to the filtrate. A blue – black or green precipitate confirmed the presence of gallic tannins or catechol tannins, respectively.

3.3.8.2 Saponins: A sample of 5 ml of each extract ether, methanol and water was vigorously shaken with 10 ml of distilled water for 2 min. The appearance of foam that persists for at least 15 min confirmed the presence of saponins.

3.3.8.3 Flavonoids: Some dilute ammonia solution 5 ml was added to a portion of 10 ml of the aqueous filtrated extract of each plant extract. This was followed by addition of 5 drops of concentrated H_2SO_4 added drop wisely. A yellow coloration observed in each extract indicated the presence of flavonoids.

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3.3.8.4 Steroids: A green coloration after addition of 0.5 ml of acetic anhydride and 0.5 ml chloroform to 1 ml of each plant extract of each sample indicated a positive test.

3.3.8.5 Alkaloids: A small portion of 0.2 ml of each extract was stirred and placed in 5 ml of 1% aqueous HCL and heated on a steam bath for 5 min. Then 1 ml of the filtrate was treated with three drops of Dragedorff's reagent. Turbidity or precipitation with these reagents is considered as evidence for the presence of alkaloids.

3.3.8.6 Sterols and terpenoids: A sample of 10 ml of each extract was placed in a small beaker and evaporated to dryness. The residue was dissolved in 0.5ml acetic anhydride and 0.5 ml chloroform. The solution was transferred into a dry test tube and 2 ml concentrated sulphuric acid was added. Brownish red or violet rings at the zone of the contact with the supernatant and green or violet coloration denoted the presence of sterols.

3.3.9 Quantitative determination of phytochemicals; phenols, flavonoids and tannins in the water extracts of the three indigenous plants

3.3.9.1 Total phenol content

The spectrophotometric method was used to determine the amounts of phenols

in the sample (Edeoga *et al.*, 2005). A sample of 2 ml of the plant water extract was defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into 50 ml flask, and then 10 ml of distilled water was added. This was followed by addition of 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylaclcohol. The samples were made up to mark and left to react for 30 min for colour development. The absorbance of the plant water extract was measured at 505 nm.

3.3.9.2 Flavonoid content

The flavonoid content was determined using the extraction method (Boham and Kocipai-Abyazan, 1994). Extraction of 10 ml of the plant water extract with 100 ml of 80% aqueous methanol at room temperature was repeated three times. The composite solution was filtered through whatman filter paper No. 42. The filtrate was later transferred into a crucible and evaporated to dryness in a water bath and weighed to constant weight.

3.3.9.3 Tannin content

Tannin content was determined by spectrophotometric method (Van-Burden and Robinson, 1981). A sample of 0.5 ml of each plant water extract was weighed

into a 50 ml plastic bottle. This was followed by addition of 50 ml of distilled water and the mixture was shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. A sample of 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.0008 M potassium ferrocyanide. The absorbance was measured at 515 nm within 10 min.

3.4 Testing for the antioxidant activity of the water extracts of the three indigenous plants

3.4.1 Quantification of prooxidation elements (Cu and Fe) in the water extracts of the three indigenous plants

Metallic elements (Copper and Iron) were analyzed using wet digestion method (Gamez *et al*., 1999). This was followed by taking readings against standard solutions using Atomic Absorption Spectroscopy (Shimadzu, AA–6200 Atomic absorption Spectrophotometer). All determinations were done in triplicate.

3.4.2 DPPH radical scavenging of the water extracts of the three indigenous plants

The scavenging effect on DPPH radical was determined by spectrophotometric method (Yen and Duh, 1993) and (Cuendet *et al.,* 1997). Approximately of 5, 10 and 15 µL of the water extracts were mixed with 5 ml of 0.004% methanolic

solution of DPPH. The DPPH solution was freshly prepared daily, stored in a flask, covered and kept in the dark at 4°C between the measurements. Each mixture was incubated for 30 min in the dark and the absorbance of the sample was read at 515 nm using the UV-visible spectrophotometer. A control consisting of distilled water was similarly prepared. The scavenging effect was evaluated in comparison with synthetic antioxidant BHA. The scavenging effect percentage of DPPH was calculated according to the following equation:

% Scavenging effect = absorbance (control) – absorbance (sample) x 100

Absorbance (control)

3.4.3 Reducing power activity of the plants water extracts of the three indigenous plants

The reducing power of the water extracts was determined by spectrophometric method (Yen and Duh, 1993). Approximately of 5, 10 and 15 μL of the water extracts were mixed with 2.5 ml of phosphate buffer of 200 mM and pH 6.6 and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C. After incubation, 2.5 ml of 10% TCA was added to the mixtures, followed by centrifugation at 650 x g for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700 nm. The reducing power was evaluated in comparison with synthetic antioxidant BHA. All determinations were done in triplicate.

3.5 Microbial analyses of the water extracts of the three indigenous plants and fermented milk containing different plants extracts

3.5.1 Changes in microbial quality of the fermented milk containing different plants extracts

The aerobic plate count technique method 966.23 (AOAC, 1995) was used to determine total plate count using nutrient agar. For general lactic acid bacteria counts MRS agar was used in anaerobic conditions. The microbial counts were carried out for 11 days. This was done to compare the quality properties of the five fermented milk samples.

3.5.2 Determination of antimicrobial activity of water extracts of the three indigenous plants

Pure cultures of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 22923) and *Candida albicans* (ATTC 90028) at *E. coli* 1.8×10^6, *S. aureus* 2.2×10^8 and *C. albicans* 2×10^5 were used for this study. Sterile disposable plates were used and appropriate media for each microorganism shown in Table 2 was prepared and poured into sterile disposable plates according to AOAC method 966.23 (AOAC, 1995). The appropriate plates were then inoculated with the 0.1 ml of each pure microorganisms was uniformly spread on the plates.

Table 2: Test microorganisms, their appropriate media, incubation time and
temperature

Microorganisms	Appropriate media	Incubation time and temperature
Candida albicans (ATTC 90028)	Potato Dextrose Agar with 10% tartaric acid	25 °C for 5 days
Escherichia coli (ATCC 25922)	Violet-Red Bile Glucose Agar	37 °C for 24 h
Staphylococcus aureus (ATCC 22923)	Baird Parker with egg yolk Tellurite	37 °C for 24 h

The estimation of linear diameter of the inhibition zone was done using the cork
and bore diffusion method with some modifications (Bauer *et al*., 1966, Barry *et
al*., 1985 and Rojas *et al*., 2003). Using a sterile cork-borer of 6 mm diameter,
holes were made into the set agar containing the bacterial culture. A 0.1 ml
portion of each water extracts was poured into the wells. A negative control of
0.1 ml of sodium metabisulfate of 40 mg/ml was used.

The plates were incubated at appropriate temperature for appropriate time as shown in Table 3. This was followed by measurement of the inhibition zones using a ruler after 24 h for bacteria and 5 days for fungi. Antimicrobial activity was recorded if the zone of inhibition was greater than 9 mm (Hassan *et al.*, 2006).

3.5.3 Determination of Minimum inhibitory concentration (MIC) of the water extracts of the three indigenous plants

Minimum inhibitory concentration was determined using the standard method with modifications (Wariso and Ebong, 1996). Plates that showed significant inhibitory activity of more than 9 mm inhibitory diameter on the test microorganisms were used for this test. Nutrient broth (OXOID, UK) was used for this test; 1ml of the prepared broth was dispensed in to the test tubes numbered 1-12 using a sterile pipette. A sample of 1ml plant water extract at concentration of 40 mg/ml was dispensed into each of the tubes numbered 1 and 2. Subsequently from tube 2, dilution was carried out by transferring 1ml to the next tube to tube number 10 and 1ml from tube number 10 was discarded. Tube 11 was control for sterility of the medium and tube 12 for viability of the organisms.

Pure bacterial cultures *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 22923) at concentrations of *E. coli* $1.8\text{x}10^6$, *S. aureus* $2.2\text{x}10^8$ and *C. albicans* $2\text{x}10^5$ concentration was used for this study. From these concentrations, 0.1 ml of the inoculum was transferred into each of the tubes 2-12 with the exception of tube 11. The final concentration of the water extracts in each of the test tubes numbered 1-10 after dilution were 40,000; 20,000; 10,000; 5,000; 2,500; 1,250; 625; 312.5,156.25, 78.125 µg/ml, respectively. The tubes were incubated at 37 ºC for 24-48 h and examined for growth. The last tube in which growth failed to occur was the MIC tube.

3.5.4 Survival of microorganisms in fermented milk containing different plants extracts

The survival of microorganisms in fermented milk preserved using the three water extracts at a concentration of 40 mg/ml was carried out according to a method described by Uhart with slight modifications (Uhart *et al.,* 2006). However, prior to fermentation, 0.1ml of pure cultures of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 22923) and *Candida albicans* (ATTC 90028) at a concentration of *E. coli* $1.8\text{x}10^6$, *S. aureus* $2.2\text{x}10^8$ and *C. albicans* $2\text{x}10^5$, were added to 5 ml of each milk sample. Samples were mixed at 260 rpm for 1 min to distribute the inoculum. The milk samples were left to ferment naturally at ambient temperature for 3 days. At test time-after three days

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of fermentation, 1ml of each milk sample was diluted using 9 ml buffered peptone water (OXOID, UK) and appropriate growth media for each microorganism was used in plating. Colonies were counted after 24 h incubation time for bacteria and 5 days for fungi. All the tests were repeated three times.

3.6 Toxicity test for the water extracts of the three indigenous plants

The toxicity test was done according to the brine shrimp lethality test (Meyer *et al*., 1982). Concentrations of the water extracts of 40,000; 20,000; 10,000; 5,000; 2,500; 1,250; 625; 312.5,156.25, 78.125 µg/ml were prepared by serial dilution in different sterile vials. A sample of 2 ml of each concentration was selected and placed in sterile vials. Each solution was made to 10 ml by adding artificial seawater and then 10 brine shrimps were transferred into each vial. The experimental vials were maintained under illuminated conditions. Controls were placed in 10 ml of artificial seawater without the water extracts. Each assay was repeated three times and the average number of surviving larvae after 24 h was recorded. Lethality concentrations (LC_{50} Values) for each assay were calculated by taking average of the three experiments and subjecting the data to probit analysis, SAS program version 8.2.

3.7 Sensory quality assessment of the fermented milk containing different plant extracts

Sensory quality assessment tests were carried on the five fermented milk samples using a 20 untrained member panel in JKUAT Food Processing Workshop. These were done to evaluate the preference and acceptability of the five fermented milk samples. A guided structured sensory evaluation questionnaire (Appendix 2) was provided and ranking was done using the hedonic scale (ASTM, 1968). Sensory attributes checked included taste, texture, aroma, colour and general appearance.

3.8 Statistical analysis

The three indigenous plants (*Lippia javanica, Olkingiri* and *Olea europaea)* and their water extracts were analyzed as treatments. The data were analyzed by ANOVA, using the Least Significant Difference test to evaluate differences among averages and the Statistical Analysis System (SAS program version 8.2 SAS Institute Inc, Cary NC USA 2002-2003). A p value < 0.05 was considered to be significant.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Plants collection and identification

The indigenous plants collected and identified are as shown in Table 3. These indigenous plants are incorporated in milk fermentation. The plants used depend on the availability. The plant part used is charred and applied on the inside surface of the gourd used for milk fermentation.

Three indigenous plants namely (*Lippia javanica, Olkingiri* and *Olea europaea*) are used in processing and preserving milk and from the discussion; incorporation of these plants in milk can extend the shelf life of fresh milk to four days without the milk souring, this milk can even be used in preparation of tea. However, after the four days the milk ferments and it can be stored for a month without spoilage.

Two plants were botanically identified however, *Olkingiri* is awaiting identification. These indigenous plants were mostly trees and shrubs. The plant parts were sourced in such a way that the part was dismembered from the plant and the plant was left to continue growing. The plants were sourced throughout the year and they were collected mostly in the evenings.

Table 3:   Three indigenous plants commonly used in milk processing and preservation by the Maasai community in Kajiado district

LOCAL NAME	BOTANICAL NAME	FAMILY	PLANT TYPE	PART USED	LOCAL USE
Osinon	*Lippia javanica (Burm. F.) Spreng*	Verbenaceae*	Shrub	stem	Applied to milk gourd before milk fermentation
Olorien	*Olea europea* ssp.africana (Mill)	Oleaceae⁻	Tree	root	Applied to milk gourd before milk fermentation
Olkingiri	Pending identification	Pending identification	Shrub	stem	Applied to milk gourd before milk fermentation

* Nairobi National Museum of Kenya   ⁻ (Maundu, *et al*., 2001)

4.2 Chemical composition of the three indigenous plants

4.2.1 Moisture, pH and ash content of the three indigenous

The moisture, pH and ash content on dry weight basis of the three plants differed significantly from one plant to another at $p<0.05$ as indicated in Table 4. The differences in the chemical composition of the plants may be related to botanical differences among the plants as shown in Table 3.

Table 4:   Moisture, pH and ash content of the three indigenous plants

Plant name	Moisture (%)	pH	Ash content (%)
Lippia javanica	13.50 ± 0.06^a	6.61 ± 0.01^b	6.89 ± 0.02^a
Olkingiri	8.84 ± 0.02^c	9.90 ± 0.01^a	6.77 ± 0.06^b
Olea europaea	22.80 ± 0.02^b	4.90 ± 0.01^c	3.20 ± 0.01^c

Mean values ± SD n=3. Means followed by similar letter in a column are not significantly different at $p<0.05$.

The moisture content ranged from 8.84 – 22.80%. *Olea europaea* had the highest moisture content 22.80% while *Olkingiri* had the lowest moisture content 8.84%. Moisture content is important in the pricing and transportation of herbs due to weight and quality factors. Drying of the plant parts is necessary to

reduce the weight and for preservation of the samples. The knowledge of the moisture content can be applied in the plant parts handling after harvesting them from the mother plant. The plants with high moisture content require stringent handling techniques to avoid spoilage especially by the microorganisms.

The pH of the plant water extracts ranged from 4.90 to 9.90. Two of the plant water extracts were acidic while *Olkingiri* water extract had alkaline pH of 9.90. This pH range is important in food processing and preservation because micro-organisms can grow and multiply only within a certain pH range. Most prefer to live in a neutral environment around pH 7.

The ash content ranged from 3.20% to 6.89%. The highest ash content 6.89% was from *Lippia javanica* while the lowest 3.20% was from *Olea europaea*. The higher ash content may be related to higher mineral content. The high ash content contributes to high ash content of the food processed with the plant material.

4.2.2 Mineral content of the three indigenous plants

All the plant materials had a reasonable amount of minerals expressed in dry weight basis as shown in Table 5.

Table 5:   Mineral composition of the three indigenous plants

Mineral element	Mg	Ca	Zn	Cu	Fe
	mg/100 g	mg/100 g	µg/100 g	µg/100 g	mg/100 g
Plant name					
Lippia javanica	4.3 ± 0.03^a	90.0 ± 0.06^a	26.4 ± 0.01^a	28.7 ± 0.01^a	0.1 ± 0.01^b
Olkingiri	1.8 ± 0.01^c	19.3 ± 0.01^b	BDL	3.1 ± 0.04^c	0.8 ± 0.01^a
Olea europaea	2.7 ± 0.01^b	20.3 ± 0.01^b	10.8 ± 0.01^b	12.2 ± 0.01^b	0.1 ± 0.01^b

Mean values $\pm$ SD n=3. Means followed by similar letter in a column are not significantly different at $p < 0.05$. BDL – Below detectable levels.

Calcium was the highest mineral ranging from 19.3 mg/100 g to 90.0 mg/100 g. This was followed by magnesium ranging from 1.8 mg/100 g to 4.3 mg/100 g. Zinc and iron was also present in considerable amount. However, in *Olkingiri* stem, zinc content was below detectable levels. *Lippia javanica* had relatively high mineral content in comparison to the other two plants (*Olkingiri* and *Olea europaea)*; this is advantageous because it may contribute to high mineral content in the fermented milk processed with its water extract. *Olea europaea*

had higher mineral content than *Olkingiri*. *Olkingiri* had higher ash content than *Olea europaea*, more investigations should be carried out to find which other minerals are found in this plant which would have lead to this occurrence.

This considerable amount of minerals in the plants is a good indication that the plants may be good sources of the vital minerals required in human nutrition. The high concentration of these minerals is advantageous since certain inorganic mineral elements (zinc, calcium) play important roles in the maintenance of normal glucose tolerance and in the release of insulin from beta cells of islets of Langerhans (Choudhary and Bandyopadhyay, 1999).

These indigenous plants may be of significance in human health when their materials are added to the milk during fermentation and then the fermented milk is consumed because the minerals they contain may also increase the mineral content in the fermented milk.

4.2.3 Qualitative phytochemical composition of the three indigenous plants

The results in Table 6 show that, the three indigenous plants contained phytochemicals namely tannins, saponins, flavonoids, alkaloids, steroids, sterols, terpenoids, reducing compounds and flavone aglycones.

The three extracting reagents had varied extracting potential in different plant parts. The best extracting solvent was methanol. Most phytochemicals were present in methanol extract, followed by water extract and lastly petroleum ether extract. The most abundant phytochemicals were tannins, they occurred in all plants and in all solvents shown in Table 6.

Tannins were found in all three extracts. The presence of tannins in these plant materials is significant in food preservation because tannins have important roles such as potent antioxidants (Trease *et al*., 1983). Besides being antioxidants, tannins have antimicrobial activity, they have been found to form irreversible complexes with proline-rich proteins (Hagerman and Butler, 1981).

Saponins were only present in the water extracts of *Lippia javanica* and *Olea europaea.* However saponins were absent in *Olkingiri* in all extracts. Saponins are natural antioxidants (http://en.wikipedia.org/wiki/saponin) and as natural phytomicrobial agents they elicit antibacterial, antifungal and antiviral effects.

Sterols and terpenoids were present in most plant extracts except in *Olkingiri* water extract. Terpenes or terpenoids are active against bacteria (Tassou *et al*., 1995), fungi (Suresh *et al*., 1997).

Table 6:   Qualitative phytochemical composition of the three indigenous plants

Plant name	T	S	F	A	ST	SE-TE	R C	F A
Lippia javanica								
Water	+	+	+	-	+	-	+	-
Methanol	+	-	+	+	+	-	+	-
Ether	+	-	+	-	+	+	-	-
Olkingiri								
Water	+	-	+	-	-	-	-	-
Methanol	+	-	+	+	-	+	+	+
Ether	+	-	-	-	+	-	-	-
Olea europaea								
Water	+	+	+	-	+	+	-	-
Methanol	+	-	+	+	-	+	+	+
Ether	+	-	+	-	+	+	-	-

T=Tannins   F=Flavonoids   SE=Sterols   TE=Terpenoids

S=Saponins   A=Alkaloids   ST=Steroids   FA= Flavone aglycones

RC=Reducing compounds

+= Present   -=Absent

Flavonoids were found in all water and methanol extracts of the three plants. However, flavonoids were absent in ether extract of *Olkingiri*. Some of the most commonly occurring polyphenols are the flavonoids with a large number of phenolic hydroxyl groups attached to ring structures that confer the antioxidant activity. They are multifunctional, acting as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers (www.activin.com/FoodPres.htm).

Lippia javanica did not have flavone aglycones in any of the three extracts. *Olkingiri* and *Olea europaea* had flavone aglycones only in methanolic extracts. Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The phenolic fraction of plant extracts has been linked to their antioxidant capacity and antimicrobial activity (Proestos *et al*., 2006). Alkaloids were only found in methanolic extracts of all the three plants.

Steroids were present in all the ether extracts. *Lippia javanica* had steroids in all the three extracts. However, they were absent in *Olea europaea* methanol extract and *Olkingiri* water and methanol extracts. Reducing compounds were present in all plants tested mostly in the methanol extracts except for *Lippia javanica* where they were present both in water and methanol extracts.

The growing interest in the substitution of "traditional food preservatives," both antimicrobials and antioxidants, by natural preservatives has fostered research on plant sources and the screening of plant materials in order to identify new components. Studies on phytochemicals have showed that, plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999).

Antioxidants are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food (Zallen *et al.,* 1975). These preservatives include ascorbic acid, propyl gallate, tocopherols, tertiary butylhydroquinone, butylated hydroxyanisole and butylated hydroxytoluene (Iverson, 1995). However, potential health hazards of synthetic antioxidants in foods, including possible carcinogenicity, have been reported several times (Ford *et al.,* 1980).

4.2.4 Quantitative estimation of crude phytochemical constituents in the three indigenous plants

The results of quantitative estimation of crude phytochemicals on dry weight basis are as shown in Table 7.

Table 7:   Total phenols, tannins and flavonoids of the three indigenous plants

Plant water extract	Phenols (mg/g)	Tannins (mg/g)	Flavonoids (mg/g)
Lippia javanica	20.73±0.03[b]	1.29±0.02[c]	12.72±0.03[a]
Olkingiri	77.73 ±0.01[a]	1.34±0.02[b]	10.80±0.02[c]
Olea europaea	23.62±0.03[b]	1.52±0.03[a]	11.39±0.03[b]
LSD	0.01	0.01	0.01

Mean values ± SD n=3.Means followed by similar letter in a column are not significantly different at $p < 0.05$. LSD – Least significant difference.

The three indigenous plants had total crude phenol ranging from 20.73 mg/g–77.82 mg/g. *Olkingiri* had the highest phenol content of 77.82 mg/g while *Lippia javanica* of 20.73 mg/g. Phenolic groups play an important role in antioxidant activity (Huang and Frankel, 1997).

The tannin content of the three indigenous plants ranged from 1.29 mg/g-1.52 mg/g. The highest tannin content was found in *Olea europaea* of 1.52 mg/g and the lowest amounts in *Lippia javanica* of 1.29 mg/ml. Tannins have an important role as potent antioxidants (Trease *et al*., 1983) and in addition they have antimicrobial activity and they have been found to form irreversible complexes with proline-rich proteins (Hagerman and Butler, 1981).

There was significant difference in flavonoid content in the three indigenous plants commonly used by the Maasai community in milk preservation. The highest amounts of flavonoids were found in the *Lippia javanica* of 12.72 mg/g. Some of the most commonly occurring polyphenols are the flavonoids with a large number of phenolic hydroxyl groups attached to ring structures that confer the antioxidant activity. They are multifunctional, acting as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers (www.activin.com/FoodPres.htm).

4.3 Antioxidant activity of the water extracts of the three indigenous plants

4.3.1 Prooxidant elements (Cu^{2+} and Fe^{2+}) in the water extracts of the three

   indigenous plants

The results in Table 8, show that Fe^{2+} content was higher than Cu^{2+} content in all the water extracts. The Cu^{2+} content was not significantly different in all the extracts however, the Fe^{2+} content was significantly different at $p<0.05$. The highest amounts of Fe^{2+} of 8.99ppm were found in *Olkingiri* while the lowest amounts were found in *Lippia javanica* of 6.91ppm.

Table 8: Prooxidant elements Cu^{2+} and Fe^{2+} in the water extracts of the three

   indigenous plants

Plant water extracts	Cu^{2+}	Fe^{2+}
	ppm	ppm
Lippia javanica	0.60 ± 0.01^a	6.91 ± 0.01^c
Olkingiri	0.67 ± 0.01^a	8.99 ± 0.01^a
Olea europaea	0.60 ± 0.01^a	7.26 ± 0.01^b
LSD	0.01	0.03

Mean values $\pm$ SD n=3.Means followed by similar letter in a column are not significantly different at $p<0.05$. LSD – Least significant difference.

The antioxidative properties of the phenolic compounds are influenced by the presence or absence of metal catalysts such as copper and iron (Frankel, 1993). The presence of these metals not only acts as prooxidant but also binds the antioxidant compounds rendering them less active against oxidation of the system being tested. Therefore, the high Cu^{2+} and Fe^{2+} content in *Olkingiri* may have lowered its antioxidant activity as these elements bind on phenolic compounds lowering their antioxidant activity.

4.3.2 Scavenging effect and reducing power of the water extracts of the three indigenous plants

All the extracts showed antioxidant activity expressed as percentage scavenging effect and reducing power. However none was as high as synthetic antioxidant BHA. All the activity increased as concentration increased, but none attained the level of activity observed in BHA even at the highest concentration. Increase in the antioxidative activity with increasing concentration has been exhibited by (Singh, 2005). There was significant difference between the three plant water extracts tested at $p<0.05$. These results are shown in Table 9.

Olea europaea had the highest scavenging effect at all concentrations. The antioxidative activity may be attributed to phytochemicals in the extracts. This high scavenging effect in *Olea europaea* may be attributed to the presence of

sterols and terpenoids in this extract which were absent in the other two plants extracts.

Table 9:   Antioxidant activity of the water extracts of the three indigenous plants

Plant water extract	% Scavenging effect			Reducing power		
	5 µL	10 µL	15 µL	5 µL	10 µL	15 µL
Lippia javanica	28.6 ± 0.02^c	28.9 ± 0.02^c	32.1 ± 0.01^c	1.6 ± 0.01^a	1.6 ± 0.01^a	1.7 ± 0.01^b
Olkingiri	8.5 ± 0.01^d	11.7 ± 0.03^d	20.8 ± 0.01^d	1.4 ± 0.01^b	1.4 ± 0.01^b	1.5 ± 0.01^d
Olea europaea	38.9 ± 0.03^b	46.1 ± 0.01^b	84.0 ± 0.01^b	1.2 ± 0.01^c	1.3 ± 0.01^c	1.6 ± 0.01^c
BHA	96.7 ± 0.03^a	97.7 ± 0.01^a	98.4 ± 0.01^a	1.6 ± 0.01^a	1.6 ± 0.01^a	1.8 ± 0.01^a
LSD	0.05	0.03	0.02	0.01	0.01	0.02

Mean values $\pm$ SD n=3.Means followed by similar letter in a column are not significantly different at $p<0.05$. LSD – Least significant difference.

It has been reported that most natural antioxidative compounds often work synergistically with each other to produce broad spectrum of antioxidative activities that creates an effective defense system against free radical attack (Lu and Foo, 2001). The compound 2, 2'-diphenyl-1-picrylhydracyl (DPPH) is a free radical and has been widely used to test the free radical scavenging ability of various samples (Hatano *et al.*, 1997). The activity of phenolics and other bioactive compounds in inhibiting autooxidation in various food and biological systems as well as radical scavenging has been attributed to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice-Evans *et al.*, 1995).

Lippia javanica had the highest reducing power in all concentrations in comparison to other water extracts. The reducing power of these extracts might be a result of the hydrogen-donating ability (Shimada *et al.*, 1992) which is generally associated with presence of reductones (Pin-Der, 1998). The high reducing power in *Lippia javanica* may be attributed to the presence of reducing compounds which are absent in the other water extracts as shown in Table 6.

The results obtained are an indication that these plants may serve as a source of natural antioxidants. However their antioxidant potential should be tested in food systems to investigate their activity in food materials.

4.4 Antimicrobial potential of the three indigenous plants

4.4.1 Antimicrobial activity of the water extracts of the three indigenous plants

The results in the Table 10, expressed as the diameter of the inhibition zone of microbial growth, show that the three extracts possessed antimicrobial activity.

Table 10: Antimicrobial activity of water extracts of the three indigenous plants expressed as inhibition zone diameter (mm)

Plant water extract	Test microorganisms		
	C. albicans (ATTC 90028)	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 22923)
Lippia javanica	20.9 ± 0.03^b	9.0 ± 0.03^c	13.6 ± 0.03^a
Olkingiri	23.3 ± 0.03^a	12.3 ± 0.03^a	11.9 ± 0.03^b
Olea europaea	12.0 ± 0.01^c	11.5 ± 0.09^b	11.7 ± 0.03^c
Control	6.0 ± 0.03^d	6.0 ± 0.03^d	6.0 ± 0.03^d
LSD	0.12	0.10	0.12

Mean values $\pm$ SD n=3.Means followed by similar letter in a column are not significantly different at $p < 0.05$. LSD – Least significant difference.

The extracts were active against gram-positive bacteria, *S. aureus* and gram-negative bacteria, *E. coli*. They also inhibited the growth of the yeast, *C. albicans*. The most susceptible microorganism to all the three extracts was *C. albicans* and the best antibacterial activity was shown by the water extract of O*lkingiri*.

There was significant difference ($p<0.05$) in the three extracts. *Lippia javanica* had the highest antimicrobial activity against gram-positive bacteria, *S. aureus* inhibition diameter of 13.6 mm while *Olkingiri* had the highest antimicrobial activity against gram-negative bacteria, *E. coli* inhibition diameter of 12.3 mm and yeast, *C. albicans* inhibition diameter of 23.3 mm.

This antimicrobial activity may be attributed to the presence of phytochemicals especially the phenolic groups as shown in Table 6. O*lkingiri* had the highest antimicrobial activity against *C. albicans* and *E. coli,* this may be attributed to high phenol content in comparison to the other plants water extracts as shown in Table 7. This was an indication that these plants possessed antimicrobial activity which can be exploited in food systems.

4.4.2 Minimum inhibitory concentration (MIC) of the water extracts of the three indigenous plants

There was no significant difference in the three plants extracts p<0.05 for *E. coli*. *Lippia javanica* exhibited the lowest MIC of 40.03 mg/ml both for *E. coli* and *S. aureus*. The lowest minimum inhibitory concentration for *S. aureus* was 20.03 mg/ml was exhibited by *Olkingiri* and *Olea europea*. This is an indication that higher concentrations of the three water extracts were required to inhibit *E. coli* than to inhibit *S. aureus.* The results are shown in Table 11.

Table 11:   MIC (mg/ml) of the water extracts of the three indigenous plants

Plant water extract	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 22923)
Lippia javanica	40.03±0.03[a]	40.03±0.03[a]
Olkingiri	40.03±0.003[a]	20.03±0.03[b]
Olea europaea	40.03±0.03[a]	20.03±0.03[b]
LSD	0.11	0.12

Mean values ± SD n=3.Means followed by similar letter in a column are not significantly different at p<0.05. LSD – Least significant difference.

These results are similar to findings on the antimicrobial activities of medicinal plants used in south-western Nigeria where *M. indica* extract was required in higher concentrations against *E. coli* than the amount required against *S. aureus* (Akinpelu and Onakaya, 2006). This may be attributed to *E. coli* being gram negative and *S. aureus* being gram positive. Gram negative microorganisms are more resistant to antimicrobial activities than gram positive. High content of phenolic substances in *Olkingiri* as shown in Table 6 may have attributed to its high power in inhibiting *S. aureus*.

4.5 Toxicity of the water extracts of the three indigenous plants

The results in Table 12 indicate that, there was significant difference not only between the three extracts but also in all the three concentrations at $p < 0.05$. Lethality increased with increasing concentration in all the three extracts. At all concentrations *Olkingiri* had the lowest percentage lethality followed by *Lippia javanica* and lastly *Olea europaea*. This may be attributed to the fact that *Olkingiri* had only two groups of phytochemicals; tannins and flavonoids while the other two water extracts had a wider variety of phytochemicals (Table 6). *Olea europea* had a mixture of tannins, flavonoids, saponins, steroids, sterols and terpenoids while *Lippia javanica* had tannins, flavonoids, saponins, steroids, sterols, terpenoids and reducing compounds. These other phytochemicals may have affected the brine shrimps.

Table 12:   Mean percentage lethality of brine shrimps in water extracts of the

three plants and their lethal concentration LC_{50} values

Plant water extract	% Lethality				LC_{50}
	Concentration mg/ml				Concentration mg/ml
	0	5	10	20	
Lippia javanica	10.3 ± 0.6^a	45.0 ± 0.6^b	80.3 ± 0.3^a	$96.3\pm0.3\,^a$	5.5 ± 0.2^b
Olkingiri	10.3 ± 0.6^a	30.3 ± 0.3^c	77.3 ± 0.3^b	$91.0\pm0.3\,^b$	$8.3\pm0.\,5^a$
Olea europaea	10.3 ± 0.6^a	50.0 ± 0.6^a	79.3 ± 0.3^a	$96.0\pm0.3\,^a$	5.1 ± 0.1^c
LSD	0.10	1.76	1.15	1.50	1.20

Mean values $\pm$ SD n=3.Means followed by similar letter in a column are not significantly different at $p<0.05$. LSD – Least significant difference.

The LC_{50} of the three plants water extracts were significantly different. The *Olkingiri* had the highest LC_{50} while the *Olea europaea* had the lowest. This is an indication that it requires higher concentration of *Olkingiri* to cause toxicity while it requires less amount of *Olea europaea* for toxicity to occur. In

comparison to lethality studies by Mining (2008) *Euclea divinorum* dichloromenthane extract had LC_{50} 0.952 mg/ml this was an indication that these three plants extracts are less lethal than the *Euclea divinorum* dichloromenthane extract.

To ensure food safety, the toxicity of the extracts should be ascertained. A crude extract is considered active up to a concentration of 0.240mg/ml (Meyer et al., 1982). Therefore, extracts with LC_{50} concentrations above 0.240mg/ml should be considered for application to the fermented milk. All the plants extracts are above 0.240 mg/ml, therefore should be considered safe for human consumption. However, more tests should be carried out to ascertain the safety of these indigenous plants for human consumption though it is well known that the community has consumed them from time immemorial with any known adverse effect.

4.6 Quality properties of the fermented milk containing different plants extracts

4.6.1 Survival of microorganisms in fermented milk containing different plant extracts

There was significant difference in survival of microorganisms in the fermented milk with the plants extracts and the fermented milk without any treatment at

p<0.05. The microbial counts of the three test microorganisms were higher in the control sample (fermented milk without any treatment) than in fermented milk with the plants extracts as shown in Table 13.

There was significant reduction of microorganisms in fermented milk treated with the water extracts, in fermented milk treated with *Lippia javanica* it was observed that *S. aureus* had 2.73 log reduction and *C. albicans* had 0.95 log reduction at day 3 in comparison to the control at a significance level of p<0.05.

The high survival of the three test microorganisms in the control is an indication that the plant extracts may have had a significant effect on the survival of microorganisms in fermented milk. This may be attributed to the fact that the extracts contain bioactive compounds as indicated in Table 6, the bioactive compounds possess antimicrobial activity. *Lippia javanica* had the highest antimicrobial activity on *S. aureus* as indicated on Table 12; this may have contributed to reduced survival of *S. aureus* in fermented milk with *Lippia javanica*. *Olkingiri* had the highest antimicrobial activity on *C. albicans*, this may have contributed to its reduced survival in the fermented milk containing *Olkingiri*.

Table 13:   Microbial log count of surviving test microorganisms in fermented
              milk

Plant water extract	*C. albicans* (ATTC 90028)	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 22923)
Lippia javanica	2.02 ± 0.01^c	4.89 ± 0.01^d	5.01 ± 0.03^b
Olkingiri	2.01 ± 0.03^c	5.00 ± 0.03^c	5.01 ± 0.03^b
Olea europaea	2.47 ± 0.03^b	5.71 ± 0.01^b	5.01 ± 0.03^b
Control	2.75 ± 0.03^a	5.90 ± 0.03^a	5.96 ± 0.03^a
LSD	0.02	0.02	0.01

Mean values $\pm$ SD n=3.Means followed by similar letter in a column are not significantly different at $p<0.05$. LSD − Least significant difference.

**4.6.2 Chemical composition of the fermented milk containing different plant
       extracts**

The results are as shown in Table 14. There was significant difference in chemical composition of the five fermented milk samples tested at $p<0.05$.

Table 14:   Chemical composition of fermented milk containing different plant
               extracts

Treatments	Chemical composition			
	%Protein	%Fat	%Ash	%TSS
Lippia javanica water extract	4.8 ± 0.09^b	4.8 ± 0.02^a	0.8 ± 0.03^b	7.0 ± 0.09^e
Olkingiri water extract	4.6 ± 0.03^c	4.5 ± 0.23^b	0.7 ± 0.03^c	7.5 ± 0.03^d
Olea europaea water extract	4.6 ± 0.03^c	4.5 ± 0.02^b	0.6 ± 0.03^d	8.0 ± 0.09^c
Charred *Olkingiri* plant stem	4.6 ± 0.03^c	4.5 ± 0.02^b	0.9 ± 0.03^a	11.0 ± 0.09^a
No treatment	4.9 ± 0.03^a	4.5 ± 0.02^b	0.5 ± 0.01^e	10.0 ± 0.09^b
LSD	0.12	0.10	0.01	0.19

Mean values $\pm$ SD n=3.Means followed by similar letter in a column are not
significantly different at $p<0.05$. LSD – Least significant difference.

The results were within the normal range of typical composition of fermented milk (Fluckiger, 1982). There was significant difference in protein content in the five fermented milk samples it ranged from 4.6 to 4.9 %. The fermented milk without any treatment had the highest protein content of 4.9 % followed by the fermented milk with *Lippia javanica*. Further investigations should be carried to verify the increased percentage of protein in the fermented milk as compared with the naturally amounts found in the Maasai cow's milk.

The fat content ranged from 4.5 - 4.8 %. Milk treated with *Lippia javanica* was significantly different from all other milk samples and it had the highest fat content. This could be attributed to different microorganisms growing in the fermented milk samples, of which fat breaking microorganisms would be present. This would lead to different rates of breakdown of fat in different milk samples.

The ash content was highest in fermented milk with charred *Olkingiri* stem; it contained 0.84 % ash content. This may be attributed to the preparation of gourds using a charred plant stem. The charred material in the milk may have increased the ash content because as seen in Table 4, the *Olkingiri* stem had appreciable amount of ash of 6.77 %. The lowest amount of ash was found in the fermented milk without any treatment; it contained 0.60% ash content. The total

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soluble solids content was highest in fermented milk with charred *Olkingiri* stem which was 11.0%. The preparation of gourds using a charred plant stem may have contributed to this occurrence. The soluble part of the charred plant material may have caused increase of the total soluble solids in the milk treated with charred *Olkingiri* stem.

4.6.3 Total plate and lactic acid bacteria counts, pH and titratable acidity of the fermented milk containing different plant extracts

4.6.3.1 Total plate counts

Total plate counts increased constantly in all the milk samples up to the 14th day. However, there was a difference in total plate counts in the five fermented milk as shown in Figure 2.

The fermented milk samples without any treatment had the highest total plate counts of $10.00 - 11.40$ log cfu/ml while the fermented milk treated with *Olea europaea* had the lowest total plate counts of $5.05 - 8.38$ log cfu/ml at $p < 0.05$ level of significance.

All the fermented milk samples treated with the plants extracts had low total plate counts in comparison to the fermented milk samples without any treatment. The low total plate counts may be attributed to the fact that the plant extracts

possessed antimicrobial activity as shown in Table 10 which might have hindered the proliferation of microorganisms in the fermented milk.
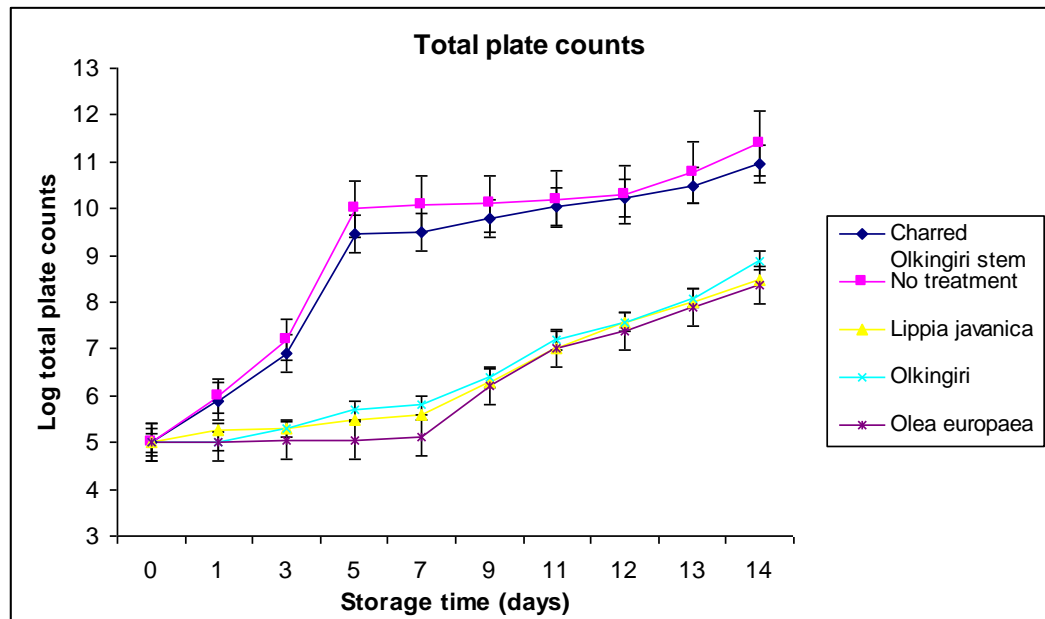
Figure 2: Total plate counts of fermented milk samples containing different plant extracts and stored at room temperature ($27\pm2°C$)

In this study the total plate counts for the fermented milk treated with plants extracts fell within the range expected in cultured milk which is $10^6 - 10^9$ (Mathaara *et al.*, 1995). However, the total plate counts for the fermented milk without any treatment were out of this range, they increased to 10^{11} on the 11th day.

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4.6.3.2 Lactic acid bacteria counts

The LAB counts increased continuously in all the samples, as shown in Figure 3. This trend is similar to studies of LAB growth as cited in microbiological quality of fermented milk produced by repeated batch culture (Nakasaki *et al*., 1998).

There was a significant difference in lactic acid bacteria (LAB) counts in the five fermented milk samples prepared with different treatments within the 11 days at $p < 0.05$ level of significance. The fermented milk without any treatment had the highest LAB counts of 9.20 -11.06 log cfu/ml in all the days in comparison to the fermented milk samples while the fermented milk with the *Olea europaea* water extracts had the lowest LAB counts of 5.00 - 7.78 log cfu/ml. This might have been attributed to the phytochemicals in the water plant extracts which might have had an influence on the growth and multiplication of LAB.

At a level of significance $p < 0.05$, the fermented milk with *Lippia javanica* 5.29 – 8.19 log cfu/ml had higher LAB than the fermented milk with *Olea europaea* 5.00 – 7.78 log cfu/ml and *Olkingiri* 5.2 – 7.79 log cfu/ml. This may be attributed to the *Lippia javanica* having the lowest phenol content 20.73 mg/g in comparison *Olea europaea* 23.62mg/g and *Olkingiri* 77.73mg/g as indicated in

Table 7 this would have lead to less inhibition of LAB by phenolic compounds in the fermented milk.

There was a significant difference of the LAB counts between the fermented milk with charred *Olkingiri* stem and the fermented milk with the water extracts.
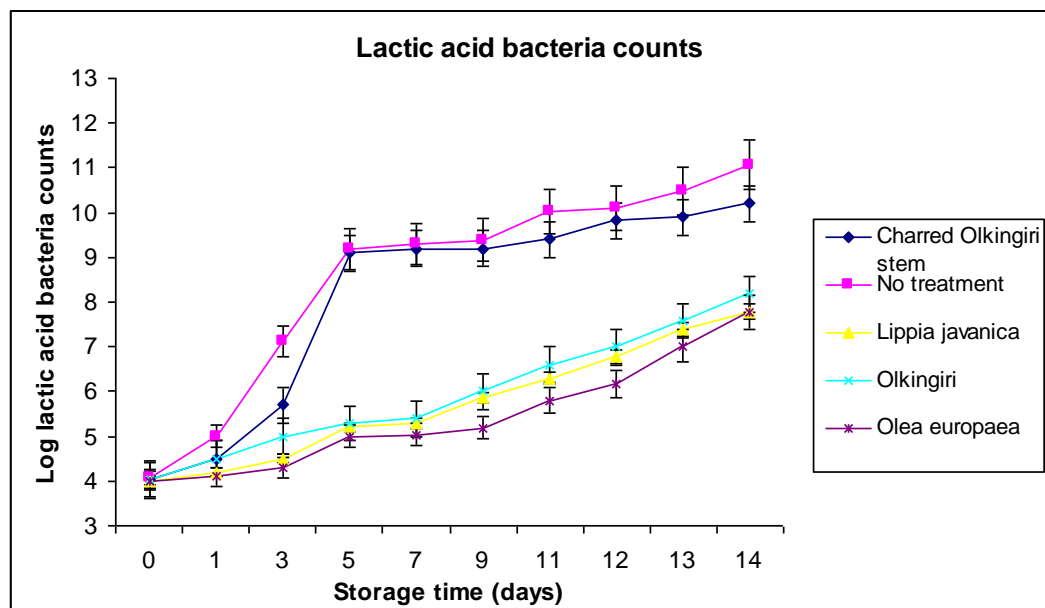
Figure 3: Lactic acid bacteria (LAB) counts of fermented milk containing different plant extracts and stored at room temperature (27±2°C)

The fermented milk with charred *Olkingiri* stem had LAB counts of 9.10 - 10.20 log cfu/ml in comparison to the fermented milk with the *Olkingiri* water extract which had LAB counts of 5.39 - 8.19 log cfu/ml at $p < 0.05$ level of significance. The direct application of the charred *Olkingiri* stem on the milk gourd might

have caused less interaction of the phytochemicals in the charred materials and the fermented milk in comparison to the interaction of the phytochemicals in the water extracts with the fermented milk. This reduced interaction might have contributed to less inhibition of LAB in fermented milk with charred *Olkingiri* stem thus less LAB counts.

4.5.3.4 Titratable acidity of the fermented milk

In this study there was continuous increase in titratable acidity in all the samples, the titratable acidity in fermented milk without any treatment increased up to 1.87% as shown in Figure 4.
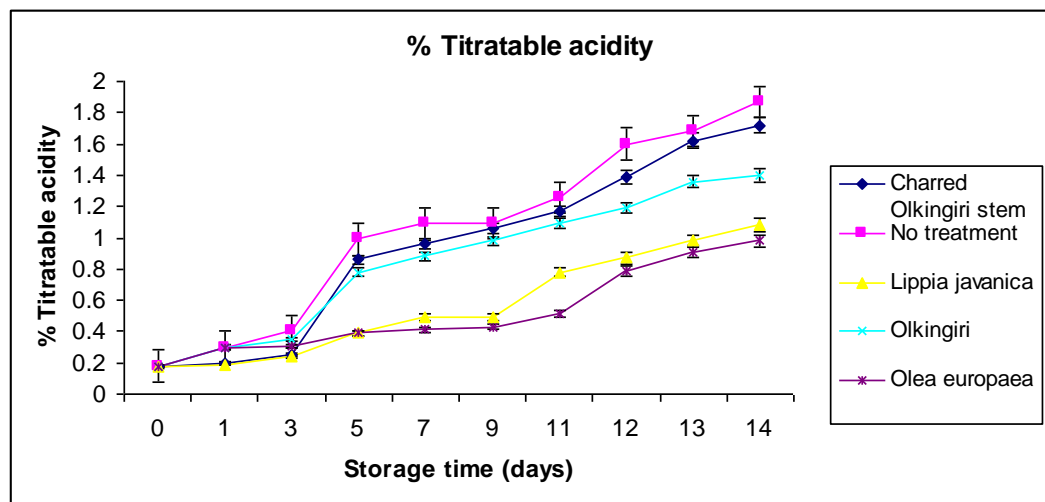

Figure 4: Titratable acidity of fermented milk containing different plant extracts and stored at room temperature (27±2°C)

The fermented milk without any treatment had the highest titratable acidity 1.08-1.87 % through out the study period in comparison to the fermented milk samples under the other conditions at a level of significance of $p<0.05$. The high titratable acidity may have contributed to high total plate counts which predominantly may have been acid producing bacteria.

The fermented milk with the plant extracts had the lowest titratable acidity of 0.41-0.98 % in comparison to the other fermented milk samples under the other conditions. This might be attributed to the phytochemicals in the water plant extracts which might have had an influence on the proliferation of acid producing bacteria thus low titratable acidity. The fermented milk with *Olkingiri* 0.78 – 1.4% had higher titratable acidity that the fermented milk with *Olea europaea* 0.41 – 0.98% and *Lippia javanica* 0.49 – 1.08% at a level of significance of $p<0.05$. This may be attributed to the high LAB counts in this milk sample which may have led to high production of lactic acid.

4.6.3.4 pH of the fermented milk

There was significant difference in pH in the five fermented milk samples stored with different treatments for 14days as indicated in Figure 5. The pH decreased continuously up to the 14th day. This might have been attributed to continuous increase in titratable acidity which might have continuously lowered the pH.

This is according to the general pathway for fermentation of milk which involves the production of lactic acid from lactose in the milk. This lowers the pH and results in a variety of products. During milk fermentation the pH usually drops to 4.3-4.5. (http://scidiv.bcc.ctc.edu/rkr/Biology201 /lectures/Respiration/ Respiration.html).
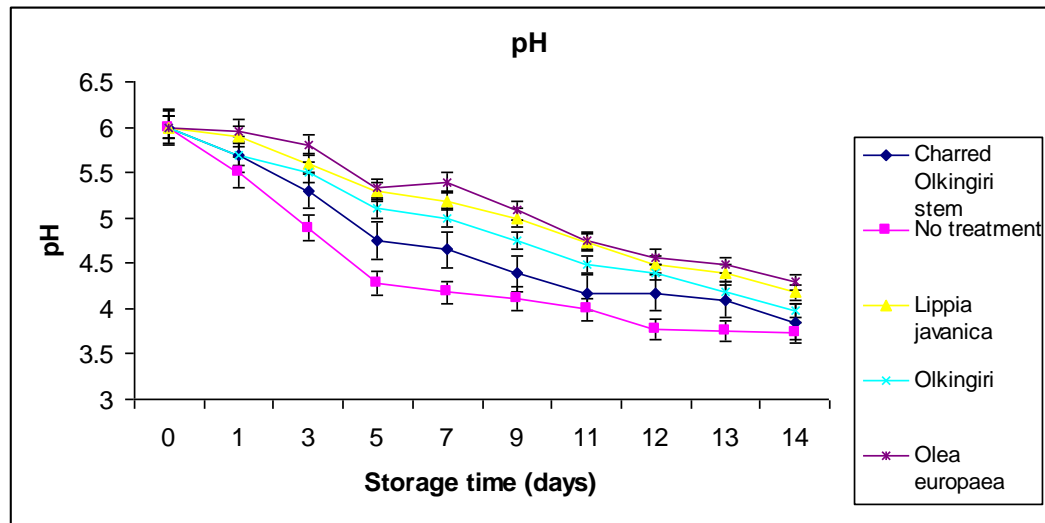
Figure 5: pH of fermented milk containing different plant extracts and stored at room temperature (27±2°C)

It was observed that the fermented milk without any treatment had the lowest pH of 4.18-3.73 all the days in comparison to all other milk samples. The fermented milk with the *Olea europaea* had the highest pH of 5.33-4.29 in comparison to the other fermented milk samples with other treatments at a level of significance

of p<0.05. This might be attributed to the phytochemicals in the plant extracts which might have lowered the proliferation of microorganisms thus reduced production of acid.

This study showed that the fermented milk with the three plants water extracts had gradual changes in all properties in comparison to the fermented milk without treatment. This may be an indication that the fermented milk with plant extracts may keep longer than the fermented milk sample without treatment. The Maasai claim that the fermented milk can be stored and be taken even after 30 days. However, from the results above it is evident that by that time the milk would be beyond the palatable stage. This claim can however be accepted on the fact that fresh milk is added continuously, most of the times daily, to the fermented milk therefore making it palatable even at that stage because of reduced pH.

4.7 Sensory quality of the fermented milk containing different plant extracts

Treatment of milk with indigenous plants significantly affected the sensory attributes this is shown in Table 15.

Table 15:   Sensory characteristics of fermented milk containing different

plant extracts

Treatments	Sensory parameters					
	Taste	Aroma	Texture	Colour	General appearance	Overall preference
Lippia javanica water extract	6.56±0.41[a]	2.94±0.23[a]	3.81±0.21[b]	3.5±0.24[b]	3.44±0.27[ab]	4.05±0.12[a]
Olkingiri water extract	6.13±0.48[a]	3.31±0.27[a]	2.50±0.26[c]	4.19±0.19[a]	3.69±0.31[a]	3.96±0.32[a]
Olea europaea water extract	5.88±0.54[a]	3.19±0.21[a]	4.69±0.28[a]	2.81±0.28[c]	2.63±0.30[c]	3.84±0.42[a]
Charred *Olkingiri* plant stem	5.19±0.67[a]	3.00±0.35[a]	4.38±0.35[ab]	1.50±0.22[d]	1.88±0.35[d]	3.19±0.10[b]
No treatment	5.50±0.47[a]	3.06±0.23[a]	4.31±0.31[ab]	2.63±0.22[c]	2.75±0.25[c]	3.64±0.20[a]
LSD	1.47	0.74	0.81	0.65	0.85	

Mean values ± SD n=3. Means followed by similar letter in a column are not

significantly different at $p<0.05$. LSD − Least significant difference.

Addition of *Lippia javanica* water extract gave the fermented milk the most preferred taste with a score of 6.56 out of 9 and the least preferred taste was from the milk fermented with charred *Olkingiri* stem which scored 5.19 points out of 9 points at $p<0.05$. The most preferred aroma 3.31 points out of 6 points was observed in fermented milk with *Olkingiri* extract while the least preferred aroma 2.94 points out of 6 points was observed in the fermented milk with *Lippia javanica* at $p<0.05$. This may be attributed to differences in chemical properties among the plant materials as shown in Table 4 and Table 6.

Fermented milk with *Olea europaea* had the most preferred texture 4.69 points out of 6 points while the least preferred texture 2.5 points out of 6 points was obtained in fermented milk with *Olkingiri* extract at $p<0.05$. This difference might have been due to the fact that the fermented milk with *Olkingiri* extract had higher titratable acidity than the fermented milk with *Olea europaea*. This high titratable acidity might have had an influence on the texture of the fermented milk making it less preferable because of curding of casein. Similar results were obtained in a study on properties of fermented milk (Zacarcheno and Massaguer-Roig, 2006), the fermented milk with the highest titratable acidity was the least preferred.

The most preferred colour 4.19 points out of 6 points and general appearance 3.69 points out of 6 points was observed in fermented milk with *Olkingiri* extract at $p<0.05$. The least preferred colour 1.5 points out of 6 points and general appearance 1.88 points out of 6 points was from fermented milk with charred *Olkingiri* stem at $p<0.05$. This may be attributed to fact that the fermented milk with charred *Olkingiri* stem had black particles from the charred material. Milk with black particles is not common in many communities and this may have contributed to less preference by the panelists who came from various communities.

Fermented milk with *Lippia javanica* had the highest overall preference 4.05 points out of 6 points while the lowest overall preference was from the fermented milk with charred *Olkingiri* stem 3.19 points out of 6 points at $p<0.05$. This may be attributed to the fact that *Lippia javanica* possessed more phytochemicals than the other plants extracts as shown in Table 6. Therefore, the results indicates that the plant extracts might have had an influence on the preference of the fermented milk and this may have contributed to better sensory qualities in fermented milk with plants extracts than in fermented milk without any treatment.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Most of the plants collected from the field commonly used by the Maasai community in Kajiado district in association with food were identified except *Olkingiri.*

The plants moisture content 8.84 - 22.80%, ash 3.20 - 6.69%, all the plants analyzed had varied amounts of minerals; calcium being the highest 19.3 - 90 mg/100g. Phytochemicals were present in most of the plants tested. The presence of the phytochemicals in the plants tested was significant because they possess both antimicrobial and antioxidant activity. This may form a basis of exploitation of these indigenous plants in food industry as sources of natural preservatives.

The water extracts exhibited antioxidative activity; the scavenging effect and the reducing power activity of water extracts ranged from 8-84% and 1.2-1.7 respectively. The water extracts had the potential to inhibit the test microorganisms *E. coli* – 3mm and *S. aureus* – 7.6 mm in comparison to the control. There was significant reduction of microorganisms in fermented milk

treated with the water extracts (*Lippia javanica) S. aureus* - 2.73 log reduction and *C. albicans* – 0.95 log reduction at day 3 in comparison to the control.

The chemical composition of the fermented milk with water extracts *Lippia javanica* at day 3 had higher fat content 4.8% in comparison to the control 4.5%. Sensory characteristics of fermented milk treated with plants water extracts were more preferred *Lippia javanica* 4.05 in comparison to the fermented milk without any treatment 3.64. It also had better quality properties total plate counts within the cultured milk range $10^6 - 10^9$.

The results obtained indicated that the indigenous plants possessed both antioxidative and antimicrobial activity. They were also safe for human consumption because their activity is greater 0.24mg/ml. Therefore these plants can be exploited as source of natural food preservatives.

5.2 Recommendations

The taxonomic classification of O*lkingiri* plant whose identity is still pending should be done. Further investigations should be carried out to identify and characterize the specific bioactive phytochemicals in the indigenous plants. There should be more investigations to find out which other minerals are found in these indigenous plants. The specific LAB in the fermented milk prepared

using the indigenous plants and their water extracts should also be studied and documented.

The antioxidative potential of these indigenous plants should be tested on food systems to ensure its effectiveness. The preservative potential of these indigenous plants should also be tested in food systems other than milk to identify the scope of their application in food industry.

The varied plants had different phytochemicals each with different antimicrobial and antioxidant capacity. The combined application of the three indigenous plants to fermented milk may cause the desired preservation effect and further studies should be carried out to establish the correct application levels.

Further investigations should be carried to verify the increased percentage of protein in the fermented milk as compared with the naturally amounts found in the Maasai cow's milk.

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APPENDIX 1: FIELD SURVEY STRUCTURED QUESTIONNAIRE

Name of village/area_____

Name of the respondent_____

Age of the respondent_____

Tick where appropriate

1. What are the plants commonly used in association with food in the community?

Local name of plants   **Plant part used**: Dry/ Fresh

                  Roots   Barks   Leaves   Seeds   Stem   Whole

part

1. _____ ____   _____   ____   ___   ____   ____   ____

2. _____ ____   _____   ____   ___   ____   ____   ____

3. _____ ____   _____   ____   ___   ____   ____   ____

2.   Where   do   you   acquire   the   plants?

_____

3. What method do you use in the collection of the plants?

        Uprooting _____

        Cutting leaves/stem_____

4. In which form do you use the plants?

Fresh _____

Dried_____

Smoked_____

Charcoal_____

Ashes_____

Any other method (establish)_____

5. In which season of the year do you collect the plant and what time of the day

Jan-April_____   Morning_____

May – Aug_____   Afternoon_____

Sept – Dec_____   Evening_____

6. How are the plants preserved and for how long can they be stored?_____

7. What are the necessary preparations on milk before treatment with the plants materials?

_____

_____

_____

_____

8. For how long can the milk be preserved using the preservative named above

     1 wk_____

     2 wk_____

     1 month_____

     3 months_____

     6 months_____

     1 year_____

9. Does the preservative affect the quality of food?

     Yes_____

     No_____

 If yes how?_____

10. Which are the most commonly used in processing and preservation of milk in this locality?

Local name of plants   **Plant part used:** Dry /Fresh

Local name of plants	Roots	Barks	Leaves	Seeds	Stem	Whole part
1. _____	_____	___	___	____	____	____
2. _____	____	___	__	____	____	____
3. _____	___	_____	___	__	____	___

11. Is there any addition information you would like to add which is not included in the questionnaire or any suggestion you think should be included that can improve the preservative under consideration?

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Thank you!

APPENDIX 2: SENSORY EVALUATION QUESTIONNAIRE

Name:_____ Date:_____

e-mail address_____

NB: Please read through the questionnaire before starting the sensory evaluation

1. Please taste the five fermented milk samples provided in sequence and indicate how much you like or dislike each one especially in relation to taste. Tick one option for each sample in the space provided and also fill in the number of the sample at the top of each column for all five samples

Sample no._____   **Sample no.**_____

9_____like extremely   9_____like extremely

8_____like very much   8_____like very much

7_____like moderately   7_____like moderately

6_____like slightly   6_____like slightly

5_____neither like or dislike   5_____neither like nor dislike

4_____dislike slightly   4_____dislike slightly

3_____dislike moderately   3_____dislike moderately

2_____dislike very much   2_____dislike very much

1_____dislike extremely   1_____dislike extremely

Sample no._____		Sample no._____	
9_____like extremely		9_____like extremely	
8_____like very much		8_____like very much	
7_____like moderately		7_____like moderately	
6_____like slightly		6_____like slightly	
5_____neither like nor dislike		5_____neither like nor dislike	
4_____dislike slightly		4_____dislike slightly	
3_____dislike moderately		3_____dislike moderately	
2_____dislike very much		2_____dislike very much	
1_____dislike extremely		1_____dislike extremely	

2. Evaluate the five samples for texture and rate them in the scale below. Tick one option for each sample in the space provided and also fill in the number of the sample at the top of each column for all five samples

Sample no.	_____	_____	_____	_____	_____
6. Very rough	_____	_____	_____	_____	_____
5. Rough	_____	_____	_____	_____	_____
4. Slightly rough	_____	_____	_____	_____	_____
3. Neither rough nor smooth	___	____	_____	_____	_____
2. Smooth	_____	_____	_____	_____	_____
1. Very smooth	_____	_____	_____	_____	_____

3. Please sniff the five samples and evaluate for aroma and rate them in the scale below. Tick one option for each sample in the space provided and also fill in the number of the sample at the top of each column for all five samples

Sample no.   _____   _____   _____ _____   _____

5. Extremely good aroma   ____   _____   _____ _____   _____

4. Good aroma   _____   _____   _____ _____   _____

3. Moderate aroma   _____   _____   _____ _____   _____

2. Low aroma   _____   _____   _____ _____   _____

1. No aroma   _____   _____   _____ _____   _____

4. Evaluate the five samples for colour and rate them in the scale below. Tick one option for each sample in the space provided and fill in the number of the sample at the top of each column for all five samples

Sample no.   _____   _____   _____ _____   _____

  5. Extremely good colour_____   _____   _____ _____   _____

4. Very good colour   _____   _____   _____ _____   _____

3. Good colour   _____   _____   _____ _____   _____

2. Slightly good colour _____   _____   _____ _____   _____

1. Not good colour   _____   _____   _____ _____   _____

5. Evaluate the five samples for general appearance and rate them in the scale below. Tick one option for each sample in the space provided and fill in the number of the sample at the top of each column for all five samples

Sample no.   _____   _____   _____ _____   _____

5. Extremely good   _____   _____   _____ _____   _____

4. Very good   _____   _____   _____ _____   _____

3. Good   _____   _____   _____ _____   _____

2. Slightly good   _____   _____   _____ _____   _____

1. Not good   _____   _____   _____ _____   _____

General comments

_____

_____

_____

Thank you very much for participating in this sensory evaluation.