PRESERVATIVE AND ANTIMICROBIAL POTENCY OF *Moringa oleifera* LEAVES EXTRACT IN SAANEN GOAT'S MILK

By

GEETHA CHANDRA REGAN

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

Student:

Name:

Date:

I certify that the report of this final year project entitled Preservative and Antimicrobial Potency of *Moringa oleifera* leaves extract in Saanen Goat's Milk by Geetha Chandra Regan, matric number F14A0074 has been examined and all the correction recommended by examiners have been done for the degree of bachelor of Applied Science (Animal Husbandry) with Honours, Faculty of Agro-Based Industry, University Malaysia Kelantan.

Approved by:

Supervisor:

Name:

Date:

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Geetha Chandra Regan

DEDICATION

I would like to dedicate this research project to my beloved parents for their endless support Mr. Chandra Regan and Mrs. Pathmini.

Preservative And Antimicrobial Potency Of *Moringa Oleifera* Leaves Extract in Saanen Goat's Milk

ABSTRACT

The study preliminarily showed preserving raw milk in a natural way in dairy microbiology. The shelf life of raw milk can be increased by inclusion of leaves extracts of *Moringa oleifera (*MO). The leaves extract of MO are prepared into treatment 1 (25%) and treatment 2 (50%). This present study consists of three parts. The very first part is determining the preservative and antimicrobial ability of MO extracts against microorganisms present in raw goat milk by standard plate count method at different storage period of 24, 48 and 72 hours at room temperature. The data shows that the treatments are not significantly differ with control sample which is more than $P>0.05$. The log CFU/mL of control, treatment 1 and treatment 2 are (5.95±0.18), (5.72±0.17) and (5.70±0.18) respectively. In the second part, the pH study on the treatments for 24 hours at room temperature were studied and it is significantly differ which is less than $P<0.05$ between control as (6.48±0.80), and treatment 1 and treatment 2 as (6.59±0.15) and (6.35±0.03). The third part of the study involves the Fourier Transform Infrared (FTIR) analysis for both MO extract and MO extract with milk. The present study showed that the maximum potential of MO as antibacterial and preservative was observed at treatment with 25% of MO extract. Hence, according to this study it can be concluded that MO can be a suitable natural preservative and has ability to use against the dairy spoilage microbes.

Keywords: Preserving, Antimicrobial, *Moringa oleifera*, Milk, Dairy Microbiology

Potensi Pengawet Dan Antimikrobial Daripada Moringa Oleifera Daun Ekstrak dalam susu kambing Saanen

ABSTRAK

Kajian awal menunjukkan pengawetan susu mentah secara semula jadi dalam mikrobiologi tenusu. Hayat simpanan susu mentah boleh ditingkatkan dengan memasukkan ekstrak daun Moringa oleifera (MO). Ekstrak daun MO disediakan ke dalam rawatan 1 (25%) dan rawatan 2 (50%). Kajian ini terdiri daripada tiga bahagian. Bahagian pertama adalah menentukan keupayaan pengawet dan antimikrobik ekstrak MO terhadap mikroorganisma yang terdapat dalam susu kambing mentah mengikut kaedah kiraan bakteria dalam plat pada tempoh penyimpanan yang berbeza iaitu 24, 48 dan 72 jam pada suhu bilik. Data menunjukkan bahawa rawatan tidak jauh berbeza dengan sampel kawalan yang lebih daripada P> 0.05. Log kawalan CFU / mL, rawatan 1 dan rawatan 2 masing-masing adalah (5.95 ± 0.18), (5.72 ± 0.17) dan (5.70 ± 0.18). Di bahagian kedua, kajian pH pada rawatan selama 24 jam pada suhu bilik telah dikaji dan perbezaannya jauh berbeza iaitu kurang daripada P <0.05 antara kawalan sebagai (6.48 ± 0.80), dan rawatan 1 dan rawatan 2 sebagai (6.59 ± 0.15) dan (6.35 ± 0.03). Bahagian ketiga kajian melibatkan analisis Fourier Transform Infrared (FTIR) untuk ekstrak MO dan ekstrak MO dengan susu. Kajian ini menunjukkan bahawa potensi maksimum MO sebagai antibakteria dan pengawet diperhatikan pada rawatan dengan 25% ekstrak MO. Oleh itu, menurut kajian ini dapat disimpulkan bahawa MO boleh menjadi pengawet semulajadi yang sesuai dan mempunyai keupayaan untuk menggunakan mikroba pemusnah tenusu.

Kata kunci: Pengawetan, Antimikrobial, *Moringa oleifera*, Susu, Mikrobiologi Tenusu

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

BTM	Bulk Tank Milk
STEC	shigatoxin-*Escherichia coli*
LPS	Lactoperoxidase System
FAO	Food and Agriculture Organization
LAB	Lactic Acid Bacteria
LOE	Lactoperoxidase Onion Extract
TBC	Total Bacteria Count
SPC	Standard Plate Count
NA	Nutrient Agar
TVC	Total Viable Count
CFU	Colony Forming Unit
SD	Standard Deviation
ºC	Degree Celcius
%	Percentage
Ml	Millilitre
Mm	Millimetre
Mg	Milligram
G	Grams
L	Litre
±	Plus – minus sign
<	Less than

CHAPTER 1

INTRODUCTION

1.1 Research Background

The term raw milk is defined as the normal mammary secretion that is obtained from milking without either addition thereto or extraction thereof (Sivakumar et.,al 2014). Based on the Zastempowska et al., (2016) findings European Union Legislation termed milk as the secretion of the mammary gland of farm animals and in which the milk is not heated up to more than 40ºC or undergone any other treatment that exhibit the equivalent effect. Milk is the complete nutritious food that provides almost all the nutrients necessary for growth and maintenance of human body. In the meantime, milk serves as a nutrient rich food that serves as an excellent culture medium for the bacterial growth (Castroudle, 2009). Hence, the consumption of raw milk has brought to food-borne outbreak and it is mostly due to the microbial contamination (Davis et al., 2014). However, according to Leedom, (2008) states that, consuming raw milk is considered safe because the 'good' bacteria and other specific components in the milk will destroy the pathogens. This will provide the whole nutrients for human.

The milk production of ruminants animal in worldwide can be represented as follows; in which cattle (85.4%), buffalo (11.1%), goat (2.1%), sheep (1.4%) and camel (0.2%) (Zastempowska et al., 2016). This statement made strong based on the findings of (Gerosa et al., 2012) states that in East and Southeast Asia, buffalo milk contributes about 51% half of the total production followed up by 10% of goat milk and 2.8% of sheep milk.

Based on this, we able to conclude that the milk production in ruminants are high and this subsequently tied up with the fact that milk and dairy products consumption are also increased. This is due to economic growth and urbanisation, added up with sophisticated marketing channels that have brought to the high production of milk and significantly bring changes in the daily supplementary patterns in which the milk and dairy products are being included in the diet (Gerosa et al., 2012). Milk is a perishable product and special considerations should be carried out to ensure the quality of milk and safety for the human consumption. One of the methods practiced in this research is the preservation of milk by retaining all its nutrients and extending the shelf life. The preservation refers to the process directed against spoilage that happens due to microbial actions (Haugaard et al.,2014). The preservation technique able to avoid the spoilage by influencing the lag phase and generation time of microorganisms specifically, psychotropic bacteria and improving the shelf life of food (Lucibello, 2015). Recently, there is a growing interest in using natural preservatives in preserving the enhancing the shelf life of milk from deterioration of microorganisms. Thus, antimicrobial agents such as the medicinal plant choose to be the main component in increasing the storage life of milk.

This study will be focussed on antimicrobial activity of medicinal plant *Moringa oleifera* on pathogens from goat's milk. This is because, plants have been an important source of natural products and the antimicrobial properties of plants have been investigated by a number of studies worldwide. *Moringa oleifera* species have long been recognized by folk medicine practitioners as having antimicrobial properties against some food borne bacteria.

1.2 Problem Statement

Milk and dairy products consumption in daily basis are increasing nowadays. The raw milk should be handled and stored in a very careful consideration subject to the microbial spoilage. The shelf life of a raw milk can be enhance if the milk is stored at refrigerator temperature of 4ºC, in which all the microbial activities of bacteria are slow down. In some rural areas, the insufficient facilities to store milk leads it way to the contamination of milk. Therefore, in order to increase the shelf life of milk, the method of preserving milk with natural herbs needed to be explore in giving out way preserving milk at no cost, in healthier manner without the addition of chemical preservatives. Hence, the research aims to incorporate the medicinal plant of *Moringa oleifera* in extending the shelf life of milk.

1.3 Objectives

1) To evaluate the antimicrobial activity and preservative effects of *Moringa oleifera* leaf extracts on foodborne bacteria in goat's milk.
2) To identify the total bacterial count and changes in pH present in the extracts of *Moringa oleifera* treated milk.

1.4 Hypothesis

Null hypothesis: *Moringa oleifera* will not be promising antibacterial in maintaining the quality of raw goat milk.

Alternate Hypothesis: *Moringa oleifera* will be a promising antibacterial in maintain the quality of raw goat milk (at least one significant difference in the mean).

1.5 Scope of Study

This field of dairy microbiology is important for the well-being of humans. Study area on dairy science focuses on the safety, security of consumption of dairy products. In addition to that, it also emphasize on the prevalence of food-borne diseases that are caused by microbes and food pathogenic bacteria and the impacts towards public health. Thus, it is important to have a research on this field in order to maintain the public health.

1.6 Significance of Study

Milk contamination due to improper storage conditions and prevalence of microbes has been identified as a complicated problem in dairy industry particularly in Malaysia with accompanied by low milk yield and production. In addition to this, this problem results in the economic loss due unhygienic conditions in farms, high cost in transporting milk to processing plant from farms and also in terms of financial issue. Therefore, this study would help the farmers and smallholders breeders in Malaysia and other developing countries to use the natural way in dealing with the storage of milk.

1.7 Limitation to Study

Currently, there is lack of literature review in the inclusion of *Moringa oleifera* as preservation of milk. Furthermore, the chemical constituent those are responsible for the antibacterial activity of plant and microbes that are causative agent of deterioration could not be further continued due to limited facilities.

CHAPTER 2

LITERATURE REVIEW

2.1 Milk Contamination

Raw milk is a highly nutritious dairy product that contains abundant water, nutrients and nearly neutral pH which serves as a good medium that harbours the growth of microorganisms that eventually results in the milk contamination. Several studies have been done on the prevalence of the foodborne pathogens in the bulk tank raw milk (BTM) and it is identified that the food borne disease outbreak is highly associated with the *Campylobacter*, *Salmonella Spp*, *Listeria monocytogenes*, and shigatoxin-producing *Esherichia coli* (STEC) (Oliver et al., 2009, Zastempowska et al., 2016). Moreover, in the studies made by Oliver et al., (2009) the range of microorganisms' prevalence in the BTM is summarized and stated as follows:

- *Campylobacter*: 2 – 9.2%
- *E. coli* O157:H7: 0 - 0.75%
- *Listeria monocytogenes*: 2.8 - 7.0%
- *Salmonella spp*: 0 – 11%
- *Shiga-toxin E. coli*: 2.4 - 3.96%
- *Yersinia enterocolitica*: 1.2 – 6.1%

The milk yield by the healthy cow is sterile until it exits through the mammary gland and usually contains low numbers of total bacterial count of 1000mL (Murphy et al.,1990). Hence, the contamination of raw milk start by during the milking process which begins with the production until the milk reaches the processing plant.

The udder status and the health of the dairy cow direct the passage for the entry of pathogens from udder into the milk. According to Ledenbach et al., (2009) the stress pressure put on the udder during the milking process, causes the teat canals to become more open and misshapen as time passes that lead the entry of pathogens infecting the glands.

Moreover, the health of the cow is an important key factor as the milk from the cow that infected with mastitis can be a threat to human consumption. For instance, in a study performed by Zastempowska et al, (2016) stated that milk yielded from the cow infected with clinical mastitis has changes in the appearance as it may contains flakes, clots or blood and have altered in colour and that can be immediately withdrawn. However, with subclinical mastitis, it does not show any visible difference and the milk is more likely to be contaminated with *Staphylococcus, Streptococcus*, enteric bacteria and also include mycoplasmas, yeasts and algae (Kuang et al., 2009; Orwa, 2017).

In addition to that, another major significant sources for milk contamination is the environmental contaminants that contaminate the cow, milking equipment, and the hygienic measurers maintained by the farmers (Ledenbach et al., 2009). On a study made by Orwa, (2017) list out the possible contaminants of environment such as the pre-milking and post-milking procedures, milking in a non-controlled environment that may be intact with the dust and faeces, the milking personnel hygienic.

The similar statement was supported by Tanya, (2013) in which the pathogens are enter into the milk directly through the animal and environmental sources and it is described in the diagram below:

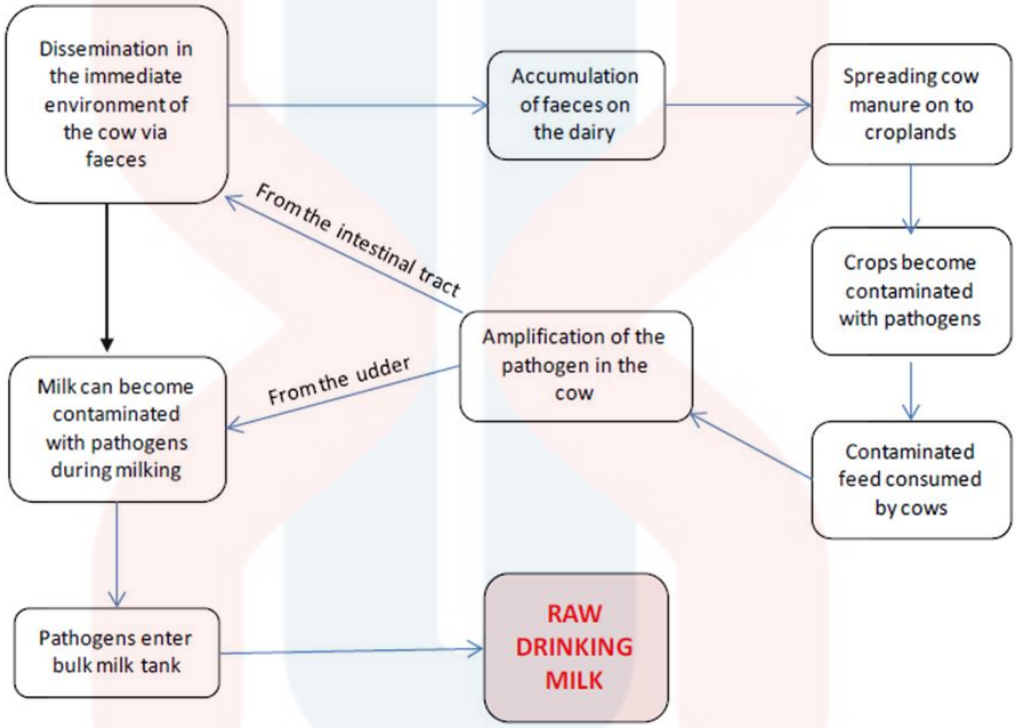


Figure 1.1: Cycling of foodborne pathogens in the dairy farm environment

The production and processing of the raw milk in farm has leads its way for the growth of microflora that spoils the dairy products. Hence, the main sources of contaminants are identified from the farm influenced by the health of the animal, environmental hygiene where the cows are housed and milked, udder status, milking techniques, disinfection of the milking machine and lastly the hygienic status of the farm attendant (Cempírková, 2007).

Together with this, dairy products that are derived from the raw milk equally give the remarkable group of the spoilage microorganisms.

Table 1.1: Dairy products and types of spoilage microorganisms (Ledenbach et al., 2009).

Food	Spoilage microorganism
Raw milk	A wide variety of different microbes
Pasteurized milk	Psychotropic, spore-formers, microbial enzymatic degradation
Concentrated milk	Spore-forming bacteria, osmophilic fungi
Dried milk	Microbial enzymatic degradation
Butter	Psychotropic, enzymatic degration
Cultured buttermilk, sour cream	Psychotropic, coliforms, yeasts, lactic acid bacteria
Cottage cheese	Psychotropic, coliforms, yeasts, molds, microbial enzymatic degradation
Yogurt, Yogurt-based drinks	Yeasts
Other fermented dairy foods	Fungi, coliforms
Cream cheese, processed cheeses	Psychotropic, coliforms, fungi, lactic acd bacteria, microbial enzymatic degradation
Ripened cheeses	Fungi, lactic acid bacteria, spore-forming bacteria, microbial enzymatic degradation

Following this, the spoilage rate for the dairy products that are readily spoiled to those that are shelf stable for months can be determined by the factors such as the moisture content, pH, processing parameters and storage temperature (Ledenbach et al., 2009).

2.2 Milk preservation

Preservation can be termed as any treatment or process of food that is applied purposely to prevent spoilage and in the meantime for keeping the storage quality of a raw material to a longer period of time than its natural keeping quality time (Rasooli, 2007). The preservatives the being used can be either natural or synthetic substances that are added to food substances in order to maintain the quality and increased shelf life (Hasan et al., 2016). The preservatives can be classified as antioxidants preservatives, antimicrobial preservatives and anti-enzymatic preservatives (Hasan et al., 2016). This method has increased over the years in the food industry in which aimed to provide healthy foods to consumers.

Furthermore, in dairy terms, the preservative for milk can be said as chemical compound or process that, when applied to milk prevents any alterations in the compositional analysis that may be caused by the growth of microorganisms (Sesķena and Jankevica, 2007). Moreover, this method could minimise the prevalence of microorganisms and as well as the chemical and physical changes in the milk. The preservation method makes the raw milk transform into stable milk products that provide a platform in maintaining its nutrients and also makes it available throughout the year. Thus, it deliberates a series of preservation techniques that enhances the quality of the milk. The preservatives required for preserving raw milk can be either chemical or organic preservatives.

2.2.1 Chemical preservatives

According to the source from the (Saha et al., 2003) it is stated that the chemical preservatives used are including of potassium dichromate, mercuric chloride, boric acid and sodium azide. These chemicals are bactericidal in nature. A similar study was conducted on the chemical milk preservation using chemical substances such as the sodium azide, bronopol, azidiol, boric acid and potassium sorbate, and the raw milk quality was tested upon the total bacteria count, somatic cell count, and fat and protein content. Based on the study, it was stated that the use of this chemical can provide stable milk quality especially bronopol (Sešķēna et al., 2007).

2.2.2 Lacteperoxidase System

In addition to that, another viable alternative source for milk preservation is through the activation of lactoperoxidase system (LPS). According to (FAO of United Nations, 2005) The LP system is one of the naturally presents inhibitory systems and it has overall bacteriostatic effect that functions as antimicrobial mechanism in the raw milk and this statement is supported by (Gaya et al., 1991 ; Zapico et al., 1991). In addition to that, the enzyme lactoperoxidase is naturally available in all mammals in adequate quantities. The quantities of the enzyme to permit the activation of the LP system varies for each animals and it is as follows, bovine milk (3 units/mL); buffalo milk (0.16-0.21units/mL); ewe milk (0.14-2.38 units/mL); and goat milk 0.05-3.55 units/mL) (Patil, 2001).

Activation of LP system is useful in maintaining the raw milk quality and it supported by the studies done by Zapico et al., (1991) whereby addition of thiocyanate and hydrogen peroxide could inhibit the growth of Gram positive bacteria such as the *Streptpcoccus* and *Bacillus* strains and as well as reduction in number of Gram negative bacteria, *Campylobacter jejuni* at ambient temperature for different subsequent hours.

A similar studies shows that, the reactivation of the LPS is possible during the storage of raw and pasteurised milk and it shows that the treated raw milk samples after pasteurization could extend the shelf life up to 11 days at 8ºC and treated pasteurized milk samples for 6 days at 16ºC (Martinez et al., 1991). Moreover, studies by Gaya et al., (1991) implies that the LPS in raw milk able to control the prevalence of *L.monocytogenes* and in the meantime extend the shelf life at refrigeration temperature of 4ºC and 8ºC.

However, the practice of activation of LPS might be effective but the sensitivity of the system explained in terms of cell wall structure of some bacteria and the different barrier properties such as the cell density and particular electron donor that depends on the concentration of hydrogen peroxide of 0.04-0.05% might be dangerous and farmers are not allowed to do this practice on their own (Punthanara et al., 2009). The LPS is a prime important industrial application in undeveloped or developing tropical countries due to the insurmountable techno-economic problems in which there is insufficient facilities for the mechanical application of refrigeration in maintaining the quality of raw milk.

2.2.3 Natural Preservatives

Natural preservatives are the technique that uses naturally available substances for the preservation of milk without the addition of any chemicals or synthetic elements. In this era, people showing more interest in naturally available herbs due to health concerns. The preservation techniques' using the herbs has been practiced by the pastoral communities long ago. This can be strongly supported with the studies done by (Nyaberi et al., 2009) in which the milk preserved with the herb *Ozoroa Insignis*. The treated milk was analysed in the parameters of titratable acidity, pH, plate and lactic acid bacteria (LAB) count within 14 days of storage and it was found that the percentage acidity and pH stabilized at 2.8% and 3.8 respectively. Followed by this, some aromatic materials such as the ginger, garlic, turmeric, betel vine and aloe vera enhanced the shelf life of both skimmed and whole milk at about 10% in a refrigerated temperature conditions for 4 days (Khusniati and Yantyati, 2008).

Moreover, Mwaura, (2014) stated that the use of plant extracts as a preservative is a preferable method. This is because, most the herbaceous plants are rich in phytochemicals and minerals that makes them the great antimicrobial and antioxidant properties in which have a tendency in substituting the chemical preservatives.

In addition to this, a closer look at the research done by the Sivakumar, (2014) have stated several techniques on using natural preservation methods. The methods includes of preservation of raw buffalo milk with the inclusion of different preservatives such as the lactoperoxidase onion extract (LOE), banana pseudo stem juice and mango seed kernel that stored at room temperature extend the storage life of the milk and reduced the total bacterial count (TBC), and inhibits the growth of *coliform*, exhibited antibacterial against *Escherichia coli.*

Furthermore, on a survey conducted by Melesse., (2014) shows that the *Olea Africana* plant material used in the Ethiopia for smoking the camel milk which in the meantime enhances the flavour, taste, improved shelf life of milk and lowers the bacterial count. Apart from the herbs, honey was used due to its natural preservative capability. The pasteurised milk samples treated with honey in concentration of 100µl in 500ml of milk exhibit the bacterial growth better when it is stored in 4ºC (Krushna et al., 2014).

2.3 *Moringa oleifera*

2.3.1 Description of *Moringa oleifera*

Moringa oleifera LAM plant belongs to the family *Moringaceae* is a native plant of India and it can be found abundantly in the region of Northern India surrounding the Sub Himalayan areas. Then, this plant widely grows and cultivated in countries like Niger, Haiti, Pakistan and China. However, now this can be greatly distributed in Philippines, Cambodia, Central America, North and South America, and also in the Caribbean Island. This tree can grow well in humid tropics and hot dry lands. It can tolerate with the minimum rainfall requirement which was estimated about 250mm and maximum about 300mm with pH of 5.0 – 9.0 annually (Anwar et al. 2007)

Moringa oleifera is known to be called in different regional names in India. It is called as Sajina ,sajna (Bengali); Sahinjan, mungna (Hindi); Murinna, Muringa, Tisnagandha (Malayalam); Sevaga, Segata (Marathi); Sohanjana (Punjabi); Sobhanjana, Sigru, Murungi, Dvishiguru (Sanskrit) and Sehjan (Urdu) (Fahey,2005). It is also called in the names of Drumstick tree, Horseradish tree and Benzolive in English. Moreover, it also called in the name of Kelor tree (Moyo and Masika, 2011).

In addition to that, there are different varieties of *Moringa* available and they are named based on their specificity. For *Moringa* that produces 60-90cm long pods are called Jaffna or Yazhpanam (Pandey, 2012). In addition to that, there are another type of *Moringa*; Chavakacheri murungai in which it produces pods of 90-120cm long. Moreover, Chemmurungai that can be found with red tipped fruits, Kadumurungai, Palmurungai, Puna murungai that has bitter taste with thick pulp and lastly with the Kodikkal murungai (Lalas and Tsakni, 2002 , Pandey, 2012).

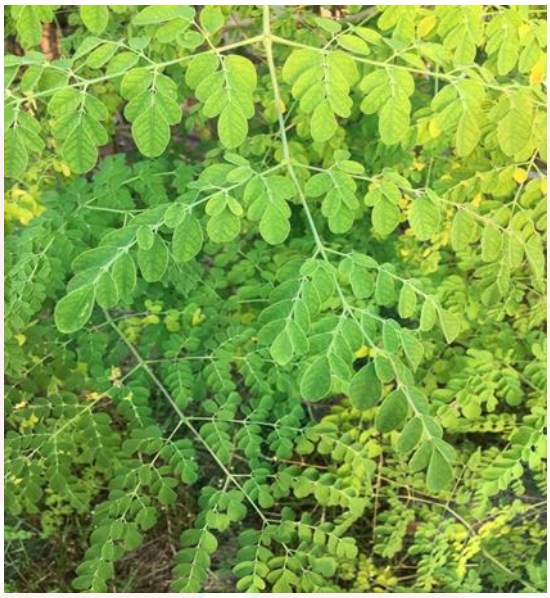


Figure 2.1 The leaves of *Moringa oleifera*.

2.3.2 Taxonomy Hierarchy

Kingdom	Plantae
Class	Magnoliopsida
Order	Brassicales
Family	Moringaceae - moringa
Genus	Moringa Adans
Species	*Moringa Oleifera*

Figure 2.2 Taxonomy Hierarchy of *Moringa oleifera.*

2.3.3 Traditional uses of *Moringa oleifera*

Moringa oleifera has been identified as a medicinal plant and it had been recognized and utilised in the Ayurvedic and Unani medicinal system long ago. This statement was supported by Al_husnan and Alkahtani (2016) who describes this tree as " tree of life " and followed by Pandey., et al (2012) that states it is one of the most amazing trees God has created. This is due to the fact that, almost every part of the drumstick tree; leaves, flowers, fruits, root, seed, bark and including the gum contains the medicinal properties. Moreover, *Moringa* tree also called as panacea whereby it could cure more than 300 diseases and had been used by Indians and Africans as medicinal plant for a long time (Gopalakrishnan et al., 2016). Previous study by Pandey et al., (2012), who stated several medicinal properties has been attributed in *Moringa oleifera*. The effective medicinal herbal formulations of *Moringa oleifera* can be attributed in terms of antipyretic, antiasthmatic, anti-inflammatory, antiarthritic and analgesic, hypocholesterolemic, antithyroid, antimircobial, anaphylactic, hepatoprotective, radioprotective, antiulcer, antipasmodic, antihyperglyemic, antitumor, antiplasmodial, antifertility and abortifacient, antioxidant and antiperoxidative, diuretic and antiurolithiatic, antihypertensive and cardio protective.

The leaves of *Moringa*, are rich in sources of proteins, vitamins, calcium and potassium (Anwar et al., 2007 ; Gopalakrishnan et al., 2016). Moreover, *Moringa* consists of vitamins that are differentiated into beta-carotene of vitamin A, folic acid, pyridoxine and nicotinic acid of vitamin B, vitamin C, D and E (Gopalakrishnan et al., 2016). In addition to that, in the same study it also stated about phytochemicals' present in *Moringa* as such tannis, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids, and as well as reducing sugar.

The leaves can be eaten fresh, cooked or stored for a long time and there will be no nutritional degradation. *Moringa oleifera* leaves are applied in the treatment of anaemia and in the irregularities of menstrual, leaf paste with curd used as internally for the stomach ache and applied externally for sprains (Pandey, 2012). Moreover, the leaf extracts has been used to combat malnutrition in small children and nursing mothers (Pandey, 2012). Looking further on this, in Philippines *M. oleifera* known to be mother's best friend' due to its properties that able to increase woman's milk production (Anwar et al., 2007).

In addition to that, treatments using the root of the tree lowers back pain, kidney pain and constipation and also used as a cardiac tonic. The flowers have been utmost effective in treating muscle diseases, tumours, and enlargement of the spleen and as abortifacient. The seed attributes high medicinal values whereby the extract helps in decreasing the liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanate (Anwar et al., 2007). A number of herbal properties can be described from various part of this miracle tree. It is extensively being used in cure many common illness of inflammation, infectious diseases in conjunction with haematological, gastrointestinal, cardiovascular diseases.

2.3.4 Antimicrobial properties of *Moringa oleifera*

One of the common properties exerted by this tree is the antimicrobial effect and various researches have been done in this study area (Pal et al., 1995 ; Bukar et al., 2010 ; Ckeresaqb et al., 1991). An aqueous extract of the leaves was tested for its susceptibility against some pathogenic bacteria such as *Eschericchia coli*, *Staphyloccus aureus*, and *Pseudomonas aeruginosa* which are known to be the major causative agents of diseases like mastitis, abortions and upper respiratory complications that results in a wider zone of inhibition (Al_husnan and Alkahtani,

2016). In study conducted by Pal et al., (1995) suggested that the antimicrobial activity of the leaf extract on gram positive, gram negative and acid fast bacteria *like B cereus, B subtilis, E-coli, Straph Aureus, S luteus* and *M phlei* revealed that, ethanolic extract found to effective against the growth of the microorganisms. It is shown that the *E-coli* showed wider zone of inhibition followed by B subtilis, then B cereus.

The use of *Moringa oleifera* does not limited as disinfectant only but the study was further continued on antimicrobial activities of the leaves against some food borne pathogens (Bukar et al., 2010). The *Moringa oleifera* ethanol leaf extract showed broadest spectrum zone of inhibition against four food-borne bacterial isolates *Enterobacter spp* (0.7mm), *S aureus* (0.8mm), *P aeruginosa* (0.7mm) and *E-coli* (0.7mm) which are found to be more pathogenic and toxigenic microorganisms that causes deterioration of food.

It was also reported that, these bacteria were sensitive at concentrations of 200mg/ml (Bukar et al., 2010). The leaf extracts of *Moringa oleifera* exert higher antimicrobial activity against Gram negative bacteria when comparing with Gram positive bacterial strains (Al_husnan and Alkahtani, 2016). In contrast to that, in another study by Peixoto et al., (2011) states that the antimicrobial activity of the leaves extract shows higher resistant against Gram positive bacteria than Gram negative bacteria.

CHAPTER 3

METHODOLOGY

3.1 Collection of sample, *Moringa oleifera*

The samples of *Moringa oleifera* were collected from Banting, Selangor. The stalks were removed and only leaves were collected. The fresh leaves were weighed and recorded. The weights of fresh leaves were 300g. The fresh leaves were washed thoroughly with tap water to remove any particles attached on it. The fresh leaves were air dried at room temperature for about 2 weeks until the leaves all are completely dried and turned into dark green colour.

The dried leaves were blend into fine particles and sieved at 0.1mm sieve. This is because different leaves have different nutritive value. Thus, by grinding and sieving the samples, we can get homogenous sample and in the meantime can prevent any biases in the experiment. The weight of powdered sample were weighed and recorded. The weights of powdered sample were 150g. Amount of moisture content of the leaves calculated as below:

$$Moisture\ content(\%) = \frac{weight\ of\ fresh\ leaves(g) - weight\ of\ dried\ (g)}{weight\ of\ fresh\ leaves}$$

$$Moisture\ content\ (\%) = \frac{300g - 150}{300g}\ x100 = 50\%$$

The moisture content obtained in the 300g of fresh leaves of *Moringa oleifera* is 50%.

3.1.1 *Moringa oleifera* extracts preparation

The total of powdered *Moringa oleifera* collected was 150g. Accurately, 20g of sample was taken from the 150g of powdered sample. The 20g sample was weighed and put in a beaker. Then, 400mL of distilled water was then added into the 20g of sample, and mixed thoroughly by using a spatula. The ratio of sample to distilled water used is 20g: 400mL, that is 20 times of dilution. This ratio is important in providing the adequate amount of water for the heating, to prevent the sample from becoming thickened.

Then, the mixture was heated at 100ºC for about 15 minutes until the mixture was slightly boiled. Direct heating method using Bunsen burner was used. A thermometer was used to check the temperature of the decocted mixture. After 15 minutes, the mixture was allowed to cool for about 30 minutes. After that, the mixture was collected in a conical flask after a thorough filtration by using sterile Whatman Filter Paper No.1. The mixture was filtered for few times, to get the full filtration of the mixture.

The collected extract of *Moringa oleifera* was 200mL. The extract was diluted by adding 200mL of distilled water. This is because the extracts should be in a same volume of distilled water used. The extracts are then transferred into two media bottles of 250mL and covered with aluminium foil. The media bottles are then stored at -4ºC. The extracts obtained and prepared are now called as 400mL of stock solution of *Moringa oleifera*.

3.1.2 Milk sample preparation

The raw milk sample was collected from a Saanen goat dairy farm, in Felda Kemahang, Tanah Merah, Kelantan. The sample collected was 1L of fresh raw milk that hand milked early in the morning and it was stored at -18ºC in a sealed condition.

3.2 Treatment preparation

Two different treatments 25% and 50% are prepared for this experiment. 25mL and 50mL of plant extract was measured in a cylinder respectively and poured into a media bottles. Then, 75mL of fresh raw goat milk was added into the media bottle containing 25% of plant extract to make a stock solution of 100mL for the treatment 1. The 25mL of extract in 100mL (25mL of plant extract + 75mL of milk) of milk makes it a treatment 1 of 25%. The same method was used to prepare the treatment 2. 50mL of plant extract was added with 50mL of fresh raw goat milk. The 50mL of extract in 100mL (50mL of plant extract + 50mL of milk) of milk makes it a treatment 2. All the treatments were replicated three times.

$$Volume = \frac{Volume\ of\ solute}{Volume\ of\ solution} \times 100$$

Stock solution of treatment 1

$$\frac{25mL}{100mL} \times 100\% = 25\%$$

Stock solution of treatment 2

$$\frac{50mL}{100mL} \times 100\% = 50\%$$

3.2.1 Treatment preparation for Standard Plate Count (SPC)

10mL of solution from stock solution of treatment 1 was measured in a measuring cylinder and poured into a test tube and labelled. The same 10mL solution from stock solution of treatment 2 was measured and poured in a test tube and labelled.

3.2.2 Treatment preparation for pH

The balance 90mL in the stock solution was agitated aseptically into three media bottles. For treatment 1, 30mL of solution was poured into a media bottle and this repeated for another two bottles and labelled. The same procedure applied for preparing treatment 2 and control.

3.3 Media preparation

Nutrient agar (NA) was used as media for this experiment. Nutrient agar is recommended in determination of plate count of microbes and in the meantime to asses total viable microbes growth of a sample. This is because, the nutrient agar rich in composition that allows the growth of more microbes for the total viable counts (TVC).

About 500mL of distilled water was measured using cylinder then poured into the conical flask. Then, 11.5g of NA and 0.41g of sodium chloride (NaCL) was weighed using electronic balance and was added into the conical flask containing the distilled water. The solution was swirled using a magnetic stirrer by placing the solution on a hot plate to ensure the agar dissolved as much as possible.

The magnetic stirrer was then taking out, and the solution was poured into a media bottle of 1000mL and covered with the lid. The agar solution was autoclaved at 121ºC for 15 minutes to sterilize the solution (written on the bottle labels).

After autoclave the solution was cooled down to temperature of 55ºC and pH was measured by using litmus papers to make sure the agar solution is neutral. The agar solution then poured into sterilized petri dishes. About 15-20mL of agar solution was poured into each petri dish. The plates are let to cool for about 15 minutes. Once the agar solidified, they were wrapped with parafilm to prevent from contamination and stored upside down. One 500mL of agar solution can yield 25 plates and the experiment was done three times to get 75 plates.

3.3.1 Microbial sampling and serial dilution.

Saline solution of 0.5% was prepared. By using a 10mL of measuring cylinder, 9mL of saline solution was dispensed into 6 measuring cylinder and labelled as 10^{-1} – $10^{-6.}$ Similarly, the petri dishes also labelled as above. For treatment 1, 1mL of sample was drawn from the test tube using a sterile 1mL pipette and transferred to test tube of 10-1 dilution factor. This gave a dilution of 1:10 dilution (1mL of sample + 9mL of saline solution).

The suspension was shaked, and 1mL of the dilution then transferred to next test tube. This gave a dilution of 1:100 (10-2) dilution of original sample. The procedure continues up to dilution factor of 10-6. Lastly, 1ml from the dilution factor 10-6 test tube discarded to give equal amount. The same procedure applied for treatment 2 and control sample and this was repeated for three times.

3.3.2 Spread plate method

For this experiment spread plate technique was used. By using a sterile pipette, 0.1mL of the culture was drawn from the solution of 10^1 onto the agar surface. The petri dish closed immediately to avoid contamination. The glass rod was dip in a 70% of Ethanol and the passed it quickly to the Bunsen Burner flame.

This is to sterilize the glass rod. After the glass rod cooled for 10 seconds, it was used to spread the agar plate. Spreading was done by lightly moving the rod back and front across the plate, working up and down. This process done until all the suspension had been spread thoroughly. Then, the plate was covered with the lid and allowed to wait until all the suspension was absorbed in the agar so that when plate is inverted the suspension will not drip off (Harley, Laboratory Exercises in Laboratory, (2014). The plate were covered with parafilm and placed inverted and incubate at 37ºC for 24, 48 and 72 hours. The process repeated for all dilution factors until 10^6. The same method applied for treatment 2 and control.

3.4 Standard Plate Count (SPC)

The total plate count was counted and recorded for every 24, 48 and 72 hours. The desired numbers of colonies (25-300) in each plate were chosen. The cell density of the original sample calculated by counting the number of colonies formed on the plate (Talaro and Chess, The Foundations in Microbiology, 2015). The total colony forming units (CFU/mL) calculated by using:

$$CFU = \frac{Colonies\ on\ plate}{volume\ of\ sample\ plated \times dilution}$$

3.5 Reading of pH

The media bottles of control, treatment 1 and treatment 2 were kept in a room temperature and pH reading was taken for every 2 subsequent hours for 24 hours.

3.6 Fourier Transferred Infrared Spectroscopy (FTIR) analysis

The FTIR analysis was done for sample, plant extract that incorporated with milk. The FTIR spectra were obtained in the wavenumber ranges from 500-4000cm^{-1} during 32 scans with 4cm^{-1} resolution using a FTIR spectrometer.

3.7 Statistical analysis

All the data the controlled and the treatments with the plant extracts were subjected to one-way Analysis of Variance (ANOVA) using Microsoft Word Excel 2013 and SPSS. The differences in mean were done by using Duncan test at ($P<0.05$). In the present study, all the values were expressed in terms of mean ± standard error (SE).

CHAPTER 4

RESULTS

In the present study, different concentration of *Moringa oleifera* leaves extract were added into the fresh raw goat milk at room temperature at a different storage time. The standard plate count (SPC) and pH were determined for each treatment. The experiment was conducted with fresh raw goat milk (experimental control), fresh raw goat milk treated with 25 % of *Moringa oleifera* leaves extract (treatment 1) and with 50% of *Moringa oleifera* leaves extract (treatment 2). An experimental control was used to compare with other treatments so that the relationship of control with treatment 1 and 2 are better understood. The FTIR analyses were done for samples of *Moringa* and *Moringa* with milk respectively.

4.1 Standard plate count (SPC)

In the present study, the standard plate counts (SPC) were determined and expressed as mean of log CFU/mL. The values presented were expressed as the (mean ± SE). The study revealed that there is no significant difference among the treatments applied on different storage time of 24, 48 and 72 hours.

4.1.1 Effect of treatment on SPC at storage period of 24 hour

Table 4.1 Average standard plate count (mean ± SE) log CFU/mL at 24 hour.

Treatments	Control	Treatment 1(25%)	Treatment 2 (50%)
Log CFU/mL	5.80±0.30[a]	5.53±0.30[a]	5.56±0.32[a]

*[a] refers to difference between treatment

* *refer to P value significantly at (P<0.05)

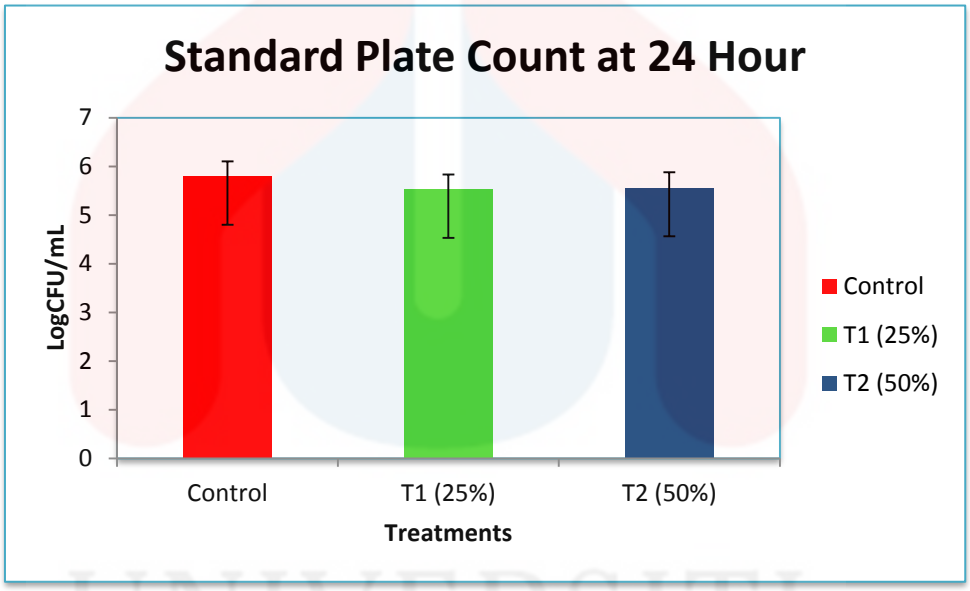


Figure 4.1 Average standard plate count Log CFU/mL at 24 hour.

The sensitivity of *Moringa oleifera* in treated milk samples were presented in Table 4.1 at 24 hours. Based on the data obtained, it shows that the log CFU/mL of control was the highest with (5.80 ± 0.30). At this hour, the sample treated with 50% of extracts exceeded the mean of sample treated with 25%.

This results as treatment 2 contains high SPC than the treatment 1 with log CFU/mL of (5.53 ± 0.30) and (5.56 ± 0.32) respectively. Thus, treatment 1 shows the least amount of viable microbes. It shows no differences among the treatments at 24 hours.

4.1.2 Effect of treatment on SPC at storage period of 48 hours.

Table 4.2 Average standard plate count (mean ± SE) log CFU/mL at 48 hour.

Treatments	Control	Treatment 1(25%)	Treatment 2 (50%)
Log CFU/mL	5.96±0.32[a]	5.72±0.30[a]	5.65±0.32[a]

*[a] refers to difference between treatment

* means (P<0.05)

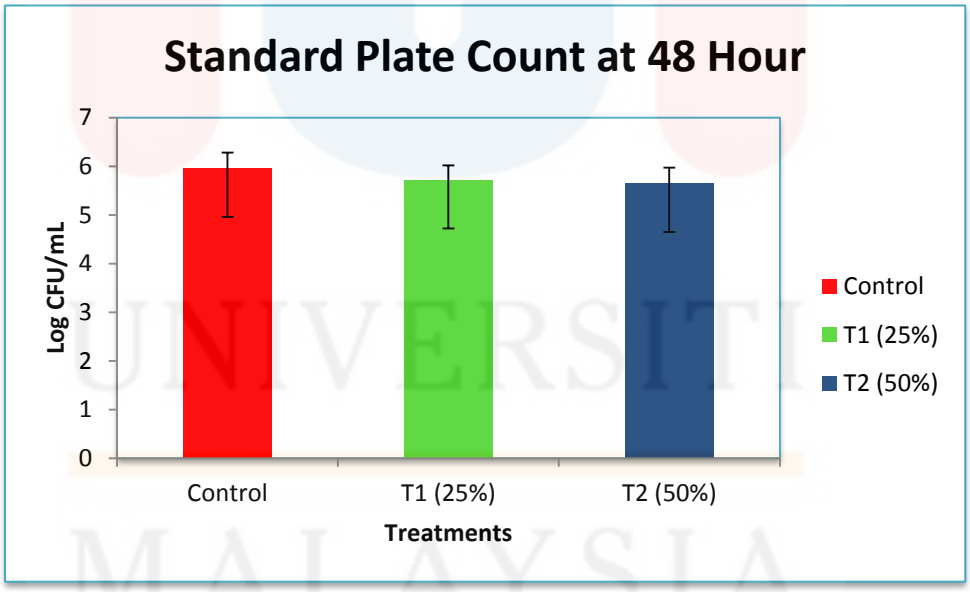


Figure 4.2 Average standard plate count Log CFU/mL at 48 hours.

The viable counts of microbes of control and treated samples during 48 hours shows no variation at significant level of (P<0.05) and the results were tabulated in the table 4.2.

The milk treated with 50% of *Moringa oleifera* shows the least number of total SPC with value of (5.65 ± 0.32) among all the treatments. The treatment 1 shows higher SPC than the treatment 2 and lower compared to control sample. The results obtained for the treatment 2 and control were (5.72 ± 0.30) and (5.96 ± 0.32) respectively.

4.1.3 Effect of treatment on SPC at storage period of 72 hours.

Table 4.3 Average standard plate count (mean ± SE) log CFU/mL at 72 hour.

Treatments	Control	Treatment 1(25%)	Treatment 2 (50%)
Log CFU/mL	6.08±0.33[a]	5.91±0.33[a]	5.88±0.32[a]

*[a] refers to difference between treatment

* *refer to P value significantly at (P<0.05)

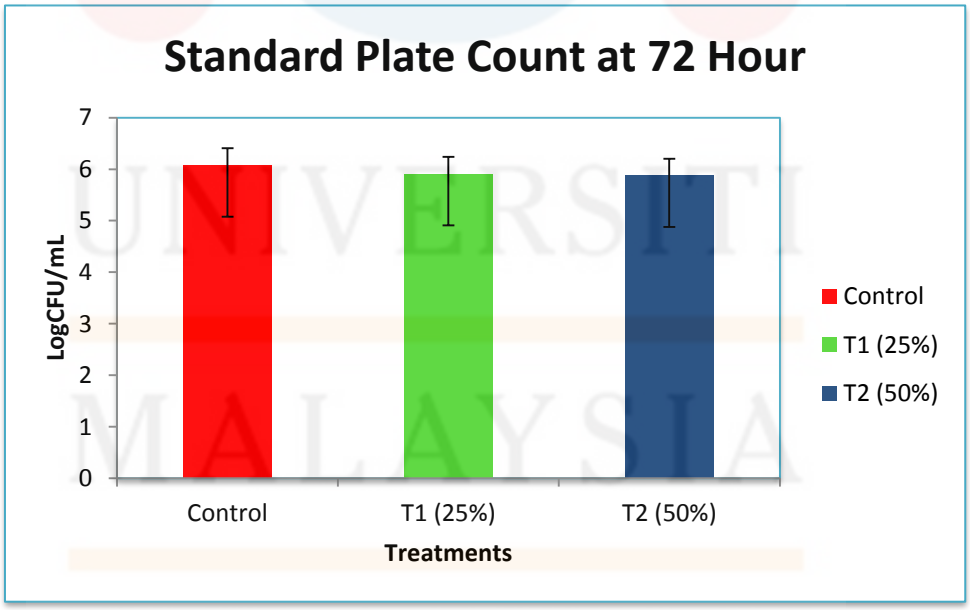


Figure 4.3 Average SPC LogCFU/mL) at 72 hours.

Table 4.3 indicates the data collected on the total SPC at storage time of 72 hours. At hour 72, the sample treated with 50% of extract shows the least number of microbial loads and it is significantly lower than the control and treatment 1. The control sample showed the highest viable counts and it is then followed by the treatment 1. The log CFU/mL values for control, treatment 1 and treatment 2 were recorded as (6.08 ± 0.33), (5.91 ± 0.33) and (5.88 ± 0.32) respectively.

4.1.4 Effect of treatment on shelf life of milk

Table 4.4 Effect of treatments on average standard plate count (mean ± SE) log CFU/mL.

Treatments	Control	Treatment 1(25%)	Treatment (50%)
Log CFU/mL	5.95±0.18[a]	5.72±0.17[a]	5.70±0.18[a]

*[ab] refers to difference between treatment

**refer to P value significantly at (P<0.05)

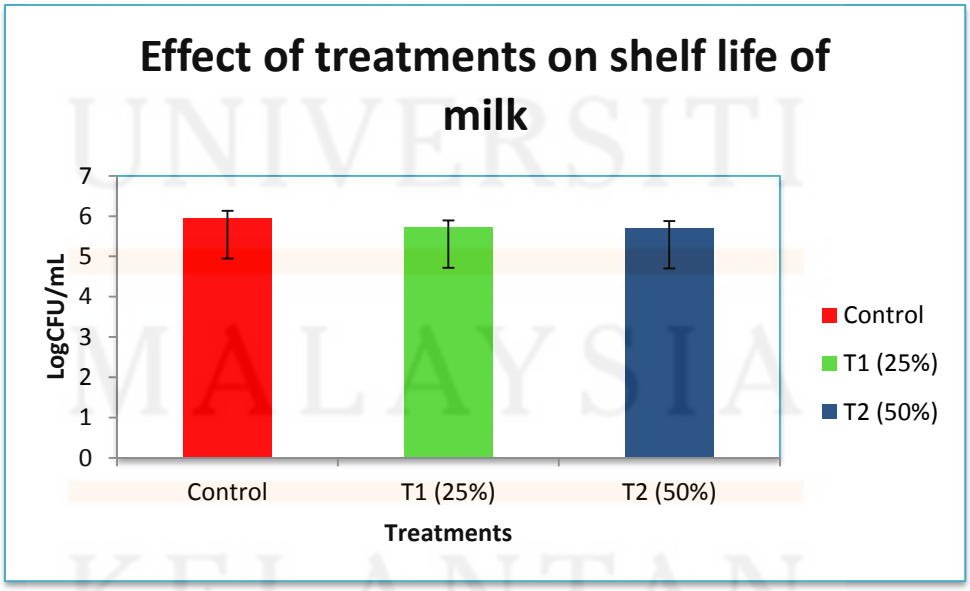


Figure 4.4 Effect of treatments on the shelf life of milk.

The effect of different treatments on the shelf life of milk at different storage period of 24, 48 and 72 hours, was determined. Based on the test, it was indicated that there were no any significant difference ($P<0.05$) among the treatments of control, treatment 1 and treatment 2. According to the data obtained in table 4.4, it shows that the there is no significant difference between the control samples and the treatments.

The control samples recorded the highest value of microbial load of (5.95 ± 0.18). Treatment 1 of 25% of extract recorded value of (5.72 ± 0.17) and the treatment 2 of 50% extract shows the least number of microbial counts among all the treatments which is (5.70 ± 0.18).

4.2 The pH value

The pH values of different treatments were determined at room temperature for every 2 hours in 24 hours. The values were expressed in mean ± standard error.

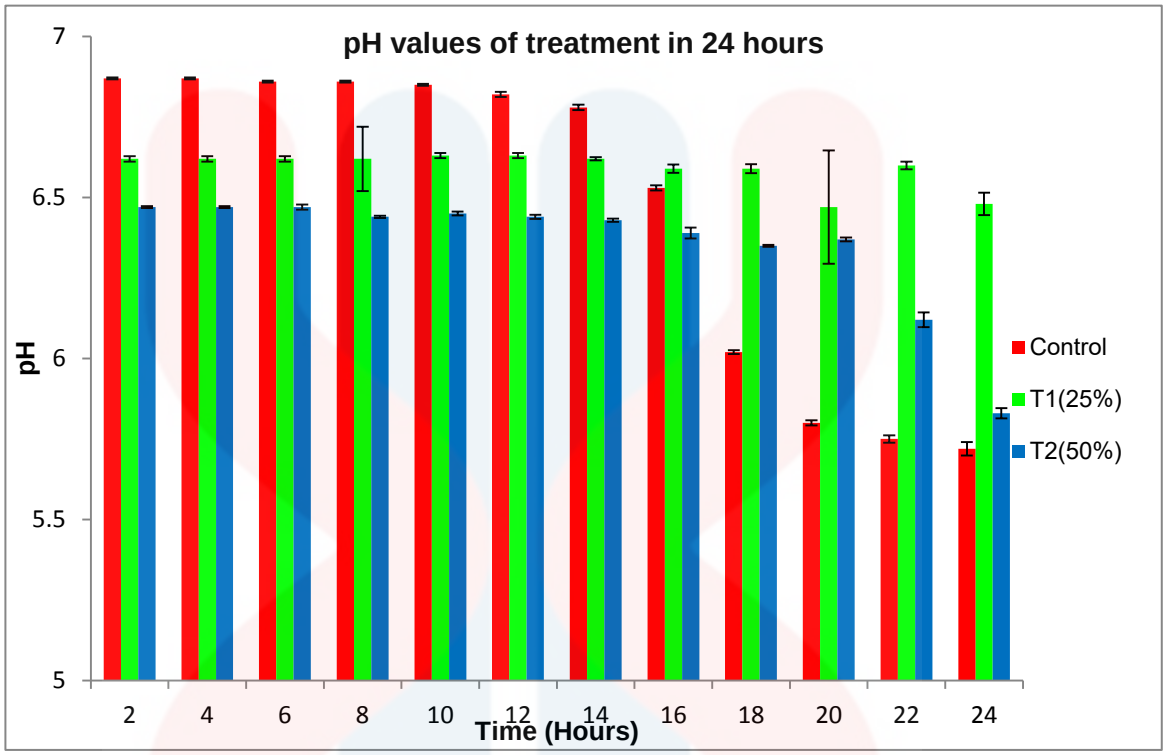
4.2.1 The pH values of different treatments in 24 hours.

Table 4.5 The pH values of treatments in 24 hour.

Storage period (hours)	Treatments		
	Control	Treatment 1	Treatment 2
1600	6.87±0.003^{a}	6.62±0.008^{b}	6.47±0.003^{c}
1800	6.87±0.003^{a}	6.62±0.008^{b}	6.47±0.003^{c}
2000	6.86±0.003^{a}	6.62±0.008^{b}	6.47±0.008^{c}
2200	6.86±0.003^{a}	6.62±0.100^{b}	6.44±0.003^{c}
0000	6.85±0.003^{a}	6.63±0.008^{b}	6.45±0.006^{c}
0200	6.82±0.008^{a}	6.63±0.008^{b}	6.44±0.006^{c}
0400	6.78±0.008^{a}	6.62±0.005^{b}	6.43±0.005^{c}
0600	6.53±0.008^{a}	6.59±0.013^{b}	6.39±0.017^{c}
0800	6.02±0.006^{a}	6.59±0.014^{b}	6.35±0.003^{c}
1000	5.80±0.008^{a}	6.47±0.176^{b}	6.37±0.006^{b}
1200	5.75±0.011^{a}	6.60±0.012^{b}	6.12±0.023^{c}
1400	5.72±0.021^{a}	6.48±0.035^{b}	5.83±0.016^{c}

*^{abc} refers to difference between treatment

* means P(<0.05)



*2=1600;4=1800;6=2000;8=2200;10=0000;12=0200;14=0400;16=0600;18=0800;20=1000;22=1200;24=1400

Figure 4.5 The pH values of treatments in 24 hour.

The pH reading for all the treatments were taken for every two hours for 24 hours at room temperature. Based on the data in the table 4.5, it shows that from 1600 hours to 0200 hours there is a significant difference at ($P<0.05$) among all the treatments. The pH values for all the treatments were almost maintained in the ranges of mean values of 6.87 to 6.82 for control; 6.62 to 6.62 for treatment 1; and 6.47 to 6.44 for treatment 2. The pH reading for the control samples were decreasing according to time. However, for treatment 1 the pH reading was not stable and fluctuating from 2200 hours 0400 hours, whereby the value increased from 6.62 to 6.63 and fall back to 6.62. Moreover, for the treatment 2, the pH value was decreasing with the time and at one point of 0000 hours the value ascent a little to 6.45 from 6.44 and fall back to 6.44 at 0200 hours.

At 1000 hours, the samples show a greater variance in pH value among the treatments and shows a significant difference at ($P<0.05$). By referring to the results, there is a significant difference between control sample and treated samples. The pH value for control sample decreases greatly from 6.02 to 5.80. Treatment 1 and treatment 2 are not differ that much. The pH values for treatment 1 fall from 6.59 to 6.47. However for treatment 2, instead of decreasing the pH value, it increased to 6.37 from 6.35. But then the value decreases greatly to 6.12 at 1200 hours. At 1400 hours, the final readings of all treatments show the variation in the pH values. The control sample and treatment 2 reaches pH values that nearly acidic which were the 5.72 and 5.83 accordingly. Treatment 1 maintained the pH value at the level of 6.48. Thus, treatment 1 shows the highest pH value and followed up by treatment 2 and control.

4.2.2 The effects of treatments on the pH values

Table 4.6: The effects of treatments on pH values.

Parameter	Control	Treatment 1	Treatment 2
pH	6.48±0.80^{ab}	6.59±0.15^{b}	6.35±0.03^{a}

* ^{ab} refer to difference between treatment within each period

*refer to P value significantly at (P<0.05)

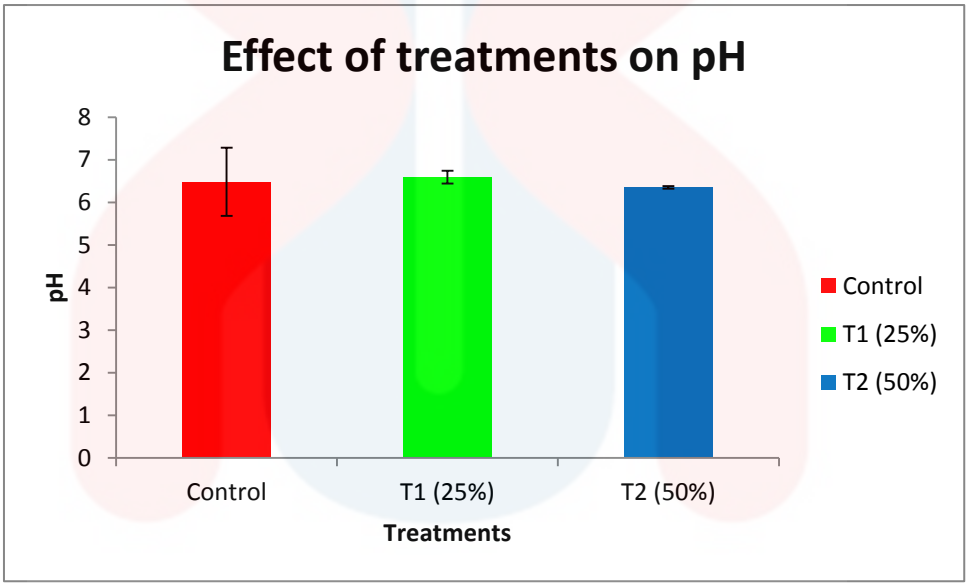


Figure 4.6 The effect of treatments on the pH values.

The table 4.6 shows the effects of treatments on the pH of the treated milk samples for 24 hours at room temperature. According to the result obtained, it shows that at significant difference of (P<0.05) there is a difference among the treatments. There is a significant difference were observed between treatments 1 and treatments 2. The control sample shows a greater variation between the treated samples.

In 24 hour room temperature storage, treatment 1 of 25% shows a higher pH value among the other treatments and it is followed up by the control and treatment 2. The pH values for the treatment 1, control and treatment 2 are (6.59 ± 0.15), (6.48 ± 0.80) and (6.35 ± 0.03) respectively.

4.3 The FTIR analysis for *Moringa oleifera*

The FTIR analysis was done for *Moringa oleifera* leaf extract and *Moringa oleifera* leaves extract fortified with milk. The figure 4.7 shows the band that appeared in the analysis for *M.Oliefera* extract and *M.OLeifera* fortified with fresh raw milk. In this analysis two significant peaks were detected and the bands that appeared in the analysis were identified as 3319.89cm^{-1} and 1636.12cm^{-1} for *M.Oleifera* extract. The peaks and band analysed for *M.Oleifera* with milk are 3319.85cm^{-1} and 1636.33cm^{-1}.

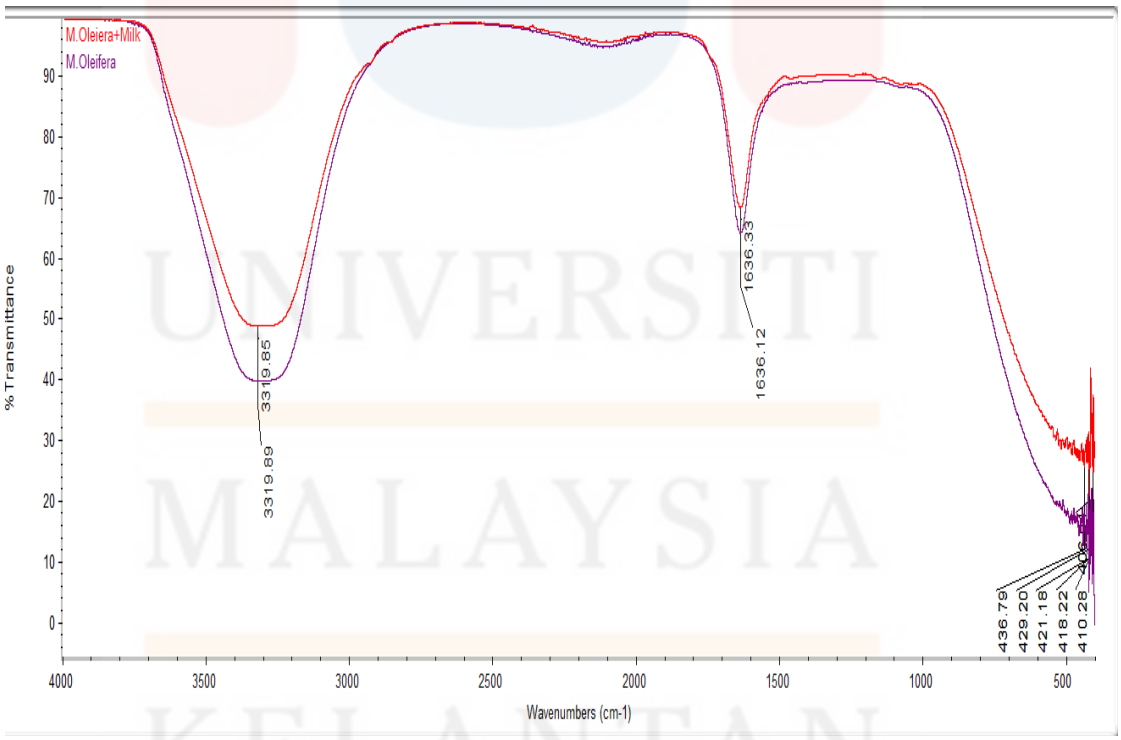


Figure 4.7 FTIR analysis of *Moringa oleifera* leaves extract in milk.

The FTIR analysis was carried out in order to identify the major components that are present when the fresh raw goat milk was fortified with the extract of *Moringa oleifera*. Both the samples of *M.Oleifera* and *M.Oleifera* with milk show the bands that are almost the same. The medium band that identified between 3319.85 cm^{-1} and 3319.89 cm^{-1} was due to the N – H stretching of alphatic primary amine of NH_2 group and it is overlapped by the strong broad band by stretching of O-H of alcohol group (Adebisi et al., 2014, Davis, 2014). The IR medium spectrum peak observed at 1636.12cm^{-1} and 1636.33cm^{-1} was due to the bending of the N – H of amine group. The peak is overlapped by the medium conjugated alkene band that results from the stretching of double bond of carbon, C =C (Cruz et al., 2012, Davis, 2014)

CHAPTER 5

DISCUSSION

5.1 Standard plate count and pH

The standard plate count for the untreated sample (control) showed a gradual increase while the treated samples of treatment 1 and 2 showed a restricted increase of microbial load at a significant difference of ($P<0.05$). By comparing the untreated samples with the treated samples, it can be concluded that the treated samples provide lower microbial load than the control sample. The treated samples however did not reduce the microbial count, but it is able to inhibit the viable growth of microbes during the storage period at room temperature. It is known that the inhibition of the extracts towards the microbes are concentration dependent (Krushna et al., 2014). It means that, as the concentration of the extracts increases, the rate of inhibition increased as well. However, there is no any significant difference at ($P<0.05$) observed between the treatments based on the table 4.4.

According to the result in table 4.5, it shows that the pH values of all treatments decreases as the time increases. In the result, it shows that there is significant difference between the control sample and treated sample. All the three treatments showed a greater variance at 1000 hours. At this hour, treatment 1 and treatment 2 shows a significant difference at ($P<0.05$) against the control sample. Followed by this, in the table 4.6, the effect of treatments on the pH in 24 hours shows that, there is a significant difference observed between control samples and treated samples.

The control sample and treatment 2 shows more acidic value and treatment 1 shows pH that are still in the consumable state. The pH value of milk samples that are below the value of 6.60 are considered more acidic (Tsioulpas et al., 2017).

There are also several similar studies conducted on the preservation ability of *Moringa oleifera. Moringa oleifera* flower pod extract that prepared from methanol and aqueous extract exhibit high antibacterial and antioxidant activity against foodborne pathogens such as *Bacillus subtitis, E-coli, S.aureus, Salmonella typht* and *Shigella* and the studies shows that, the methanol extract maintained its shelf life about 2 months in dark conditions at temperature of 4ºC (Gull et al., 2016).

Moreover, another study was conducted on the antibacterial, nutritional and sensory properties on soft cheese by inclusion of crude leaf extract of *Moringa oleifera* at different concentration of 1%, 2% and 3% by using ethanol and ether extracts (Badmos, M, & Ajiboye, 2014). Based on the findings, it is concluded that the extracts with concentration of 2% and 3% showed higher antimicrobial properties and also improved nutritional and sensory acceptability.

The antimicrobial properties of a plant can be assessed based on some other factors. For instance, according (Pasca et al., 2017) highlights in his studies that the plants with high polyphenols content are known to be possess higher antimicrobial properties. In a 100g of *Moringa* leaves it is said to be contains total polyphenols of (40.27 ± 0.9) and (36.57 ± 0.3) respectively (Chelliah et al., 2017). However, the nutritive value can be varied according to the plants and it is solely depends on the environment, genetic background and cultivation methods (Bennet et al.,2003).

The antimicrobial properties also correlate with the protein content in a plant sample. *M.oleifera* leaf extracts contains small peptides that have a defence properties against phytopathogenic fungi that are able to inhibit the growth of microbes through prevalence of bacteria from binding to chitin or by increasing the permeability of the fungal membranes (Badmos et al., 2014 , Chuang at al.,2007).

5.2 FTIR analysis of *Moringa oleifera* infused in goat milk

Based on the analysis of *M. olefera* leaves extract and *M. oleifera* extract on milk, two peaks were formed for both analysis. Formation of the band at 3319.89cm^{-1} and 1636.12cm^{-1} for *M. oleifera* exract and 3319.85cm^{-1} and 1636.33cm^{-1} for *M. oleifera* extract with milk respectively. The spectrum formed at the band of 3319.89cm^{-1} and 3319.85cm^{-1} indicates the presence of aliphatic primary amine and primary aliphatic alcohol groups. In addition to that, in the band of 1636.12cm^{-1} and 1636.33cm^{-1} functional groups such as the primary aliphatic amine and alkene groups of C = C were present.

In a study conducted by (Marcus and Nwineewii, 2015) revealed that in a band between 3500 – 3200cm^{-1} and 3500 – 3100cm^{-1} functional groups associated with the phenols and alcohols with the O – H bond and primary or secondary amine group due to stretching of N – H bond are formed in the analysis of crude extract of *Moringa oleifera*. Moreover, in the same study it states that the components present in the analysis can be either aliphatic or aromatic. Furthermore, in a *M. oleifera* analysis the mostly adsorbed functional groups are the –OH groups and C=O stretching that are seen at the band of 3280cm^{-1} and 1632cm^{-1} respectively (Solomon et al., 2017). This statement correlates with many other works (Chelliah et al., 2017, Alain et al., 2016).

In a another study by Dagnachew et al., (2013) states that in the FTIR analysis of milk protein, the adsorption bands of 1650cm^{-1} are to be consists of amide group that is due to the absorption of water. In a similar study it was stated that the O-H bending region in between of 1600-17—cm^{-1} and O-H stretching region in the bands above 3020cm-1 showed results of IR that are more or less opaque due to the high water contents in raw milk (Afseth et al., 2010).

The similar study was carried out by Nicolaou et al., (2010) and supported that at the band of 1654 – 1159cm^{-1} there are presence of amide groups of amide 1 and amide 2. Thus, based on the result obtained we are able to conclude that the major components present in the fortification of extract and milk are the amide groups due to the stretching of N-H bond.

Thus, in the present study, it was observed that the treatment 1 of 25% of *Moringa oleifera* treated sample as the most suitable concentration in preserving the raw milk. This is because, treatment 1 maintains the same inhibitory values from 24 – 72 hours of storage period which are 0.19 log CFU/mL comparing with treatment 2 that shows the variable inhibitory values at 24 hour which is 0.09 log CFU/mL and 0.23 log CFU/mL at 48 hours. Moreover, in the aspect of pH values, it is also proved that treatment 1 shows the expected outcome than treatment 2. Although, treatment 2 showed high inhibition rate, but in the aspect of pH it showed the most acidic value compared to treatment 1. This may be due to the presence of high water content in milk and *M. oleifera* extract as stated in the FTIR analysis that eventually results in the presence of more amide groups. Moreover, the drop in pH maybe due to the metabolic activities of lactic acid and other organic acid that causes the pH values to drop significantly (Adeniran and Abiose, 2011). Hence, the treatment 1 of 25% of *M. oleifera* choosed as the best treatment in extending shelf life of raw milk.

CHAPTER 6

CONCLUSION AND REFERENCE

6.1 Conclusion

In the present study, the potency of *Moringa oleifera* as antibacterial and preservative was analysed. The extract of *Moringa oleifera* leaves extract was added into the fresh raw goat milk and was analysed for the standard plate count, pH value and FTIR analysis. The present study preliminarily demonstrated that the maximum potential of *Moringa oleifera* as antibacterial and preservative was observed at treatment with 25% of *M. oleifera* extract. It is able to maintain the microbial growth and in the meantime extending the shelf life of milk. Hence, it is preferable method to use in preserving raw milk by inclusion of *Moringa oleifera* as this method safe and low cost.

6.2 Recommendations

There are several recommendations can be made on this study. First of all, further studies have to made on the antibacterial principle in the extracts of the *Moringa oleifera* that are responsible in inhibiting the growth of the spoilage microbes present in raw milk. Moreover, the bacterial identification has to be made through microbial analysis method for the microbes that are responsible in the deterioration of milk quality. Lastly, sensory evaluation has to be carried out in order to improve the present study. By considering all this recommendations, we able to come up with a better method in preserving and extending the shelf life of raw milk and in the meantime maintaining its quality

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APPENDIX

Table A.1: One way ANOVA for standard plate count at 24 hours

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.780	2	.390	.222	.801
Within Groups	89.363	51	1.752		
Total	90.143	53			

Table A.2: Post Hoc analysis using Duncan test for standard plate count at 24 hour

Duncan[a]

Treatment		N	Subset for alpha = 0.05
			1
1	Treatment	18	5.5393
2	Treatment	18	5.5663
	Control	18	5.8066
	Sig.		.573

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 18.000.

Table A.3: One way ANOVA for standard plate count at 48 hours

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.975	2	.488	.263	.770
Within Groups	94.670	51	1.856		
Total	95.646	53			

Table A.4: Post Hoc analysis using Duncan test for standard plate count at 48 hour

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
Treatment 2	18	5.6521
Treatment 1	18	5.7286
Control	18	5.9676
Sig.		.518

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 18.000.

Table A.5: One way ANOVA for standard plate count at 72 hours

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.410	2	.205	.103	.902
Within Groups	101.365	51	1.988		
Total	101.776	53			

Table A.6: Post Hoc analysis using Duncan test for standard plate count at 72 hour

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
Treatment 2	18	5.8850
Treatment 1	18	5.9177
Control	18	6.0841
Sig.		.693

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 18.000.

Table A.7: One-way ANOVA for treatments on standard plate count

LogCFUmL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.059	2	1.029	.568	.568
Within Groups	288.366	159	1.814		
Total	290.424	161			

Table A.8: Post Hoc analysis using Duncan test for treatments on standard plate count

LogCFUmL

Duncan[a]

Treatment	N	Subset for alpha = 0.05
		1
Treatment 2	54	5.7011
Treatment 1	54	5.7285
Control	54	5.9528
Sig.		.365

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 54.000.

Table A.9: One-way ANOVA for pH at 1600 hours

pH1600

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.245	2	.122	12 25.000	. 000
Within Groups	.001	6	.000		
Total	.246	8			

Table A.10: Post hoc analysis using Duncan test for pH at 1600 hours

pH1600

Duncan[a]

		Subset for alpha = 0.05		
treatment	N	1	2	3
Treatment 2	3	6.4767		
Treatment 1	3		6.6267	
Control	3			6.8767
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.11: One-way ANOVA for pH at 1800 hours

pH1800

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.245	2	.122	12 25.000	. 000
Within Groups	.001	6	.000		
Total	.246	8			

Table A.12: Post hoc analysis using Duncan test for pH at 1800 hours

pH1800

Duncan[a]

treatment	N	Subset for alpha = 0.05		
		1	2	3
Treatment 2	3	6.4767		
Treatment 1	3		6.6267	
Control	3			6.8767
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TableA.13: One-way ANOVA for pH at 2000 hours

pH2000

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.236	2	.118	707.467	.000
Within Groups	.001	6	.000		
Total	.237	8			

Table A.14: Post hoc analysis using Duncan test for pH at 2000 hours

pH2000

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Treatment 2	3	6.4733		
Treatment 1	3		6.6267	
Control	3			6.8667
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.15: One-way ANOVA for pH at 2200 hours

pH2200

		Sum of Squares	Df	Mean Square	F	Sig.
Groups	Between	.267	2	.134	10 93.455	.000
Groups	Within	.001	6	.000		
	Total	.268	8			

Table A.16: Post hoc analysis using Duncan test for pH at 2200 hours

pH2200

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Treatment 2	3	6.4467		
Treatment 1	3		6.6200	
Control	3			6.8667
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.17: One-way ANOVA for pH at 0000 hours

pH0000

		Sum of Squares	df	Mean Square	F	Sig.
Groups	Between	.241	2	.120	903.000	.000
Groups	Within	.001	6	.000		
	Total	.242	8			

Table A.18: Post hoc analysis using Duncan test for pH at 0000 hours

pH0000

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Treatment 2	3	6.4533		
Treatment 1	3		6.6333	
Control	3			6.8533
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.19: One-way ANOVA for pH at 0200 hours

pH0200

		Sum of Squares	df	Mean Square	F	Sig.
Groups	Between	.217	2	.108	541.500	.000
Groups	Within	.001	6	.000		
	Total	.218	8			

Table A.20: Post hoc analysis using Duncan test for pH at 0200 hours

pH0200

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Treatment 2	3	6.4433		
Treatment 1	3		6.6333	
Control	3			6.8233
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.21: One-way ANOVA for pH at 0400 hours

pH0400

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.188	2	.094	49.462	.000
Within Groups	.001	6	.000		
Total	.188	8			

Table A.22: Post hoc analysis using Duncan test for pH at 0400 hours

pH0400

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Treatment 2	3	6.4300		
Treatment 1	3		6.6200	
Control	3			6.7833
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.23: One-way ANOVA for pH at 0600 hours

pH0600

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.065	2	.032	57.314	.000
Within Groups	.003	6	.001		
Total	.068	8			

Table A.24: Post hoc analysis using Duncan test for pH at 0600 hours

pH0600

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Treatment 2	3	6.3933		
Control	3		6.5333	
Treatment 1	3			6.5967
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.25: One-way ANOVA for pH at 0800 hour

pH0800

	Sum of Squares	Dt	Mean Square	F	Sig.
Between Groups	.492	2	.246	922.542	.000
Within Groups	.002	6	.000		
Total	.494	8			

Table A.26: Post hoc analysis using Duncan test for pH at 0800 hours

pH0800

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Control	3	6.0233		
Treatment 2	3		6.3567	
Treatment 1	3			6.5933
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.27: One-way ANOVA for pH at 1000 hours

pH1000

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.787	2	.393	12.544	.007
Within Groups	.188	6	.031		
Total	.975	8			

Table A.28: Post hoc analysis using Duncan test for pH at 1000 hours

pH1000

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
Control	3	5.8033	
Treatment 2	3		6.3767
Treatment 1	3		6.4733
Sig.		1.000	.529

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.29: One-way ANOVA for pH at 1200 hours

pH1200

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.099	2	.549	677.274	.000
Within Groups	.005	6	.001		
Total	1.104	8			

Table A.30: Post hoc analysis using Duncan test for pH at 1200 hours

pH1200

Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Control	3	5.7500		
Treatment 2	3		6.1200	
Treatment 1	3			6.6033
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.31: One-way ANOVA for pH at 1400 hours

pH1400

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.999	2	.500	51.212	.000
Within Groups	.012	6	.002		
Total	1.011	8			

Table A.32: Post hoc analysis using Duncan test for pH at 1400 hours

pH1400

Duncan[a]

treatment	N	Subset for alpha = 0.05		
		1	2	3
Control	3	5.7233		
Treatment 2	3		5.8367	
Treatment 1	3			6.4800
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.33: One-way ANOVA for treatments on pH

ANOVA

pH

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1.016	2	.508	5.479	.005
Within Groups	9.737	105	.093		
Total	10.754	107			

TableA.34: Post Hoc analysis using Duncan test for treatments on pH

pH

Duncan[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
Treatment 2	36	6.3569	
Control	36	6.4817	6.4817
Treatment 1	36		6.5944
Sig.		.085	.119

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 36.000.

Table A.35: IR Spectroscopy Absorption Table

The following table lists **infrared spectroscopy absorptions** by frequency regions.

4000-3000 cm^{-1}						
3700-3584	medium	sharp	O-H	stretching	alcohol	free
3550-3200	strong	broad	O-H	stretching	alcohol	intermolecular bonded
3500 3400	medium		N-H	stretching	primary amine	
3400-3300 3330-3250	medium		N-H	stretching	aliphatic primary amine	
3350-3310	medium		N-H	stretching	secondary amine	
3300-2500	strong	broad	O-H	stretching	carboxylic acid	usually centered on 3000 cm^{-1}
3200-2700	weak	broad	O-H	stretching	alcohol	intramolecular bonded
3000-2800	strong	broad	N-H	stretching	amine salt	

1670-1600 cm^{-1}					
1678-1668	weak	C=C	stretching	alkene	disubstituted (trans)
1675-1665	weak	C=C	stretching	alkene	trisubstituted
1675-1665	weak	C=C	stretching	alkene	tetrasubstituted
1662-1626	medium	C=C	stretching	alkene	disubstituted (cis)
1658-1648	medium	C=C	stretching	alkene	vinylidene
1650-1600	medium	C=C	stretching	conjugated alkene	
1650-1580	medium	N-H	bending	amine	
1650-1566	medium	C=C	stretching	cyclic alkene	
1648-1638	strong	C=C	stretching	alkene	monosubstituted
1620-1610	strong	C=C	stretching	α,β-unsaturated ketone	

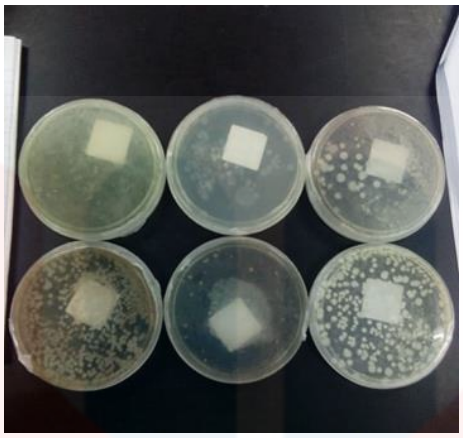

Figure A.1: The standard plate count for treatment 1 at 72 hours

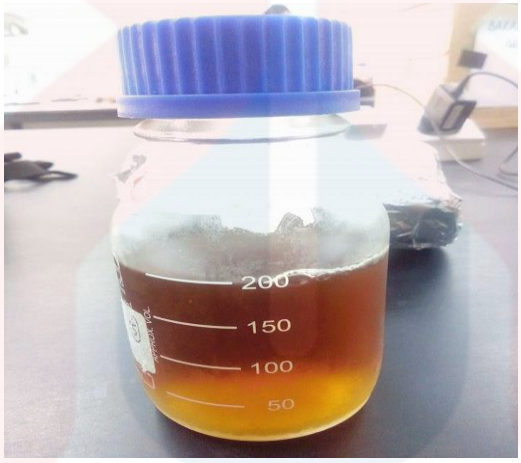

Figure A.2: The extract of *Moringa oleifera*

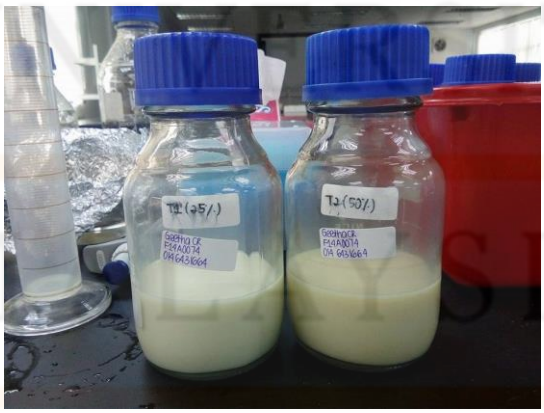

Figure A.3: The treatment 1 (25%) and treatment 2 (50%)