UNIVERSITY OF NAIROBI

COMPARISON OF THE ANTIMICROBIAL EFFECTS OF MANUKA HONEY, LOCAL HONEY, SUGAR, MOLASSES AND SILKWORM SERICINS ON COMMONLY ISOLATED WOUND MICROBES

A dissertation submitted in partial fulfillment for the award of Master of Medicine in Plastic, Reconstructive and Aesthetic Surgery.

Dr. Robert Kyalo Mbaluka

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STUDENT'S DECLARATION

I, **Dr Robert Kyalo Mbaluka**, do hereby declare that this dissertation is my original work and has not been presented for a degree in any other institution to the best of my knowledge.

Dr Robert Kyalo Mbaluka (MBChB)

H58/11364/2018

Signed: ..... Date : 1/8/2023

SUPERVISORS' APPROVAL

This dissertation has been submitted with our approval as university supervisors:

Dr Ferdinand Nang'ole

MBChB (UON), M.Med General Surgery (UON), PRAS Fellow EBOPRAS,

Consultant Plastic, Reconstructive and Aesthetic Surgeon

Unit Head, Plastic, Reconstructive and Aesthetic Surgery Unit, Department of Surgery

Faculty of Health Sciences, University of Nairobi

Signed: ..... Date: 11/05/2023

Dr. Marianne W. Mureithi

Department Chair, Senior Lecturer and Senior Research Fellow

Department of Medical Microbiology,

Faculty of Health Sciences, University of Nairobi

Signed: ..... Date: 12/5/23.

Dr. Adan Abdullahi

MBChB (UON), M.Med General Surgery (UON),

Consultant Plastic, Reconstructive and Aesthetic Surgeon

Lecturer, Department of Surgery, School of Medicine, University of Nairobi

Signed: ..... Date: 12/05/23

Dr. Angela Muoki

MBChB (UON), M.Med General Surgery (UON)

Consultant Plastic, Reconstructive and Aesthetic Surgeon

Lecturer, Department of Surgery, School of Medicine, University of Nairobi

Signed: ..... Date: 11/05/2023

DEPARTMENTAL APPROVAL

This dissertation has been presented to the University of Nairobi, Department of Surgery at a Dissertation Clinic meeting on the 30th January 2023, and is hereby approved for presentation to the Kenyatta National Hospital Ethics and Research Committee

Signed: ..... Date: 18/5/2023.

Dr. Julius Kiboi,

MBChB, M.Med Surg(UoN)

Consultant Neurosurgeon,

Chairman & Senior Lecturer, Department of Surgery, University of Nairobi

DEPARTMENT OF SURGERY
FACULTY OF HEALTH SCIENCES
P O Box 19676 - 00202 KNH
NAIROBI
Tel: 020 4915043

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

DHA –	Dihydroxyacetone
ERC –	Ethics and Research Committee
IL –	Interleukin
KAVI-ICR -	Kenya AIDS Vaccine Initiative – Institute of Clinical Research
KNH –	Kenyatta National Hospital
MGO –	Methylglyoxal
MMP -	Matrix Metalloproteinases
MRSA -	Methicillin Resistant Staphylococcus Aureus
SPSS -	Statistical Package for Social Sciences
SS –	Silk Sericin
TNF –	Tumor Necrosis Factor
UMF –	Unique Manuka Factor
UNITID-	University of Nairobi Institute of Tropical and Infectious Disease
UON –	University of Nairobi

OPERATIONAL DEFINITIONS

Manuka Honey: Honey from flowers of the native Manuka trees of New Zealand.

Unique Manuka Factor: A system of rating the potency of Manuka honey by illustrating the equivalent mass concentration of phenol (%w/v) that is needed to produce similar antibacterial activity as honey.

Local Honey: Refers to a Kenyan honey sample obtained from the National Beekeeping Institute submitted for testing by a single source producer from the Meru region, Kenya.

Sugar: Refers to Brown sugar. A sugar product that is either unrefined or partly refined and is found in crystal form. It has a characteristic brown color, due to some residual molasses.

Sugarcane Molasses: Dense, dark substance that forms the final effluent of sugar refinement and has high mineral content.

Mulberry Silkworms: The larval form or caterpillar of a silk moth that is found on mulberry trees and feeds on its leaves

Silk Sericin: One of the two proteins found in silkworm cocoons. It's the soluble protein that binds silk fibroin filaments or fibers.

Silk Fibroins: The other fibrous protein that constitutes silkworm cocoons.

Biological safety cabinet class 2 (BSC-2): A biological safety cabinet class 2 is a specialized laboratory equipment that provides operator and environmental protection during the handling of hazardous biological materials through controlled airflow patterns and HEPA filtration.

Isolate: A microbial isolate refers to a single, pure strain of microorganisms obtained from a sample, typically grown in culture, and separated from other microbial species for further study or characterization.

The McFarland standard: A reference method used to standardize the turbidity of bacterial suspensions for microbiological testing by comparing the optical density to a known standard

ABSTRACT

Study Background: Proper wound management is continually challenged by the almost inevitable installation of bacterial infection with subsequent poor prognosis and a spike in treatment costs. Despite advances in infection management using antibiotic-treated wound dressings, increased antibiotic resistance still offers a huge barrier. With the slow progress in the innovation of novel antibiotics that would otherwise overcome this challenge, the use of natural antibacterial agents such as honey, sugar and molasses offer an alternative. These agents are additionally easily available and affordable. They also seem to improve quality of patient care in the setting of chronic wounds since they are non-adherent and result in minimal pain and discomfort during change of dressing. However, comparison of their antimicrobial efficacy remains under-explored, especially in the local African and more so in the Kenyan setting despite the aforementioned.

Broad Objective: To compare the antimicrobial effects of manuka honey, local honey, sugar, sugarcane molasses and silkworm sericins on commonly isolated wound microbes.

Study Design: Controlled quasi-experimental laboratory study.

Study Site: Kenyatta National Hospital Microbiology Department and the Microbiology Department, University of Nairobi.

Participants and Methods: Bacterial and fungal isolates from KNH and UON Microbiology Department were randomly grouped into 6 study groups: Manuka honey, local honey, sugar, sugarcane molasses, silkworm sericins, and controls. Pure cultures were obtained on nutrient agar and Sabouraud Dextrose agar. McFarland standard solutions were prepared, and 100 microliters were transferred to culture bottles with test samples and controls. After incubation and streaking, MIC and MBC were determined by serial dilutions on agar plates. Findings were recorded on data collection sheets.

Data Management: Data was analyzed using SPSS version 25.0. Normality was assessed using the Shapiro-Wilk test, histograms, box plots, and Q-Q plots. Skewed data led to the use of medians and non-parametric tests. The Chi-square test compared growth rates, while the Kruskal-Wallis test examined growth rate type and presence. A p-value ≤ 0.05 indicated significance.

Results: Different growth patterns were observed with Silk Sericin and Molasses. Molasses showed increased susceptibility compared to Silk Sericin, with 64% growth of Pseudomonas versus 92% in Silk Sericin. Local honey had inferior antimicrobial effect compared to Manuka honey, with 12% growth observed versus no growth, respectively, for Pseudomonas.

Significant differences in antimicrobial susceptibility were found among the three organisms (p=0.000 for Pseudomonas, Staphylococcus, and Candida). Manuka honey and Local honey showed potent antimicrobial effects, while Molasses and Silk Sericin exhibited moderate effects. Sugar had the weakest antimicrobial effect.

Conclusions: Manuka honey and Local honey are effective against pseudomonas, staphylococcus aureus, and candida. Molasses and Silk Sericin have moderate effects, while sugar has weak antimicrobial effects. Honey-based products could be valuable natural antimicrobials for wound management, including drug-resistant infections. Our recommendation is that Local honey may be routinely used for chronic and infected wounds not responding to conventional therapy.

1.0 CHAPTER ONE: INTRODUCTION

1.1 Background

Wounds, in lower- and middle-income countries, remain associated with significant morbidity and mortality. As such, apt wound care and management ensure proper and accelerated wound healing remains critical in healthcare systems (Lj et al., 2015; Truche et al., 2021). Several local and systemic factors impede proper and acute wound healing. Of these, bacterial infections, as isolates or biofilms, form one of the key impediments to proper and timely wound healing (Guo and DiPietro, 2010). Propensity for this wound bacterial invasion increases significantly in individuals with systemic diseases for example diabetes mellitus or with associated deprivation of local factors such as arterial supply, venous drainage or concomitant wound trauma (R and Kg, 2004).

Bacterial invasion mainly affects wounds by preventing the progression of the normal inflammatory process that ensues during the wound healing process. This occurs due to sustained elevation of pro-inflammatory cytokines (interleukin-1 (IL-1), TNF-α) by bacteria and their products. The sustained elevation of these cytokines and with a subsequent increase in matrix metalloproteinases leads to either chronicity or complete failure of wound healing (Guo and DiPietro, 2010). Several antibiotics have been employed in view towards combating bacterial manifestation of wounds. This is not only due to their bacteriostatic and bactericidal effects but also from their capacity to propagate wound healing (Negut et al., 2018). Despite the successes experienced with the use of these agents, significant tolerance has been observed with several strains commonly isolated from wound infections. Additionally, use of antibiotics has been greatly associated with increased financial burden on patients as well as unavailability in resource-limited settings. In this regard, attention has shifted towards using bioresources, including herbs, minerals and animal ingredients (Shrestha et al., 2014).

Some of the most commonly used natural antimicrobials in the management of wound infection include honey and sugar dressings. Honey and sugar are frequently used in conjunction with traditional wound care techniques as adjuvant therapy. To improve the results of wound healing, healthcare providers may use topical treatments or dressings made of honey or sugar. In many regions of the world, honey and sugar are easily available and affordable solutions for wound healing. This makes them particularly useful in environments with limited resources or places where cutting-edge wound care solutions could be more difficult to find or more expensive. The therapy of diabetic foot ulcers is one instance of how honey is used in wound care. Manuka honey is a good example of medical-grade honey that is chosen for its recognized

antibacterial action (Alam et al., 2014). In the tropics, sugar is commonly utilized as a wound dressing, and is often applied in its crystallized form or as a paste (Mphande et al., 2007).

Honey has been used for medicinal purposes since ancient times due to its antimicrobial activity and positive influence on wound healing. Its bacteriostatic and bactericidal effects are concentration-dependent, and are attributed to several factors such as its hydrogen peroxide constituent, high sugar content, low pH, phytochemical agents, and chemical factors such as beeswax, propolis, nectar, pollen lysozymes, and organic acids (Mphande et al., 2007). Direct application of sugar on wounds can also promote wound healing by reducing edema, increasing bacteriostatic capacity, promoting small vessel dilation, bacterial lysis, and aiding in wound cleansing (Rahiman and Pool, 2016). Further, sugar cane molasses has recently gained interest in its use as an antibacterial and antioxidant in wounds due to its rich mineral content (Rahiman and Pool, 2016). In various studies documenting modern use, sugar has been utilized in the treatment of mediastinal wounds after cardiac surgeries, back wounds and pressure ulcers showing promising results (Pieper and Caliri, 2003)

Sericin, one of the two proteins found in the cocoon of the silkworm Bombyx mori, has physicochemical characteristics that make it a promising biomaterial for biomedical applications. It has been shown to possess antimicrobial, antitumor, antioxidant, and anticoagulant properties, as well as good oxygen permeability and moisture organizing qualities. Sericin has also been demonstrated to be immunologically inert, making it a safe biomaterial of choice with potential applications in the field of biomedicine. In addition, it has been introduced to cell lines in various culture media and has not shown any cytotoxicity.

The use of these agents has been further pronounced due to their availability, affordability, capacity to enhance the wound healing environment and their associated lack of pain during removal compared to modern dressing materials (Mphande et al., 2007). Furthermore, Aramwit et. al. in 2013 showed that the topical application of a silver sulfadiazine and 8% silkworm sericin on patients with burn wounds showed accelerated wound healing, reduced pain. Despite the aforementioned, the efficacy and degree of impact of these natural antimicrobials on wound healing remains under-explored, especially in Africa.

This study, therefore, aimed to explore the antibacterial effects of Manuka honey, local honey, sugar, sugarcane molasses and silkworm sericins and compare their effect on commonly found wound microorganisms.

1.2 Problem Statement

The accessibility, affordability, and ability to improve the conditions for wound healing, when compared to traditional dressing materials, have all led to the increased usage of natural antimicrobial substances. Despite the aforementioned, research on the effectiveness and extent of the effects of these antimicrobial substances on wound healing is still lacking, particularly in the context of developing countries of the African region. Therefore, the objective of this study was to investigate the antibacterial properties of Manuka honey, local honey, sugar, sugarcane molasses, and silkworm Sericins and to compare these properties' impact on common wound pathogens.

1.3 Study Justification and significance

Proper wound management continually remains critical in ensuring proper and accelerated wound healing as well as averting associated morbidity and mortality (Lj et al., 2015). Microbial infection in wounds is almost inevitable and presents a couple of consequences including delays in wound healing, prolongation of hospital stay, intensification of trauma, increased disarticulation/amputation risks, and further worsens treatment costs (Kassam et al., 2017).

Antibiotic resistance is still a challenge in the management of wounds. This has led to poor patient outcomes and astronomical costs in the management of wounds. Antibiotic resistance has also been worsened by the lack of innovations of novel antibiotics that would otherwise overcome the challenge with antimicrobial resistance. As a result, the use of natural agents with antibacterial effects such as honey, sugar, molasses and silk sericins offer refuge (Mphande et al., 2007; Rahiman and Pool, 2016; Scagnelli, 2016, Aramwit 2013). These agents are easily available, affordable and also improve quality of patient care especially in chronic wounds since they are non-adherent and do not often require replacement (Negut et al., 2018). Their compared antibacterial efficacy however remains under-explored especially in the local African setting despite the aforementioned.

The study's findings helps further understand the antimicrobial effects of Manuka and local Kenyan honey, sugar, sugarcane molasses and silkworm sericins. These findings should advice for their use in routine wound management. The use of these agents will allow wound management to become more accessible and more affordable. Current research on the antimicrobial effects of natural agents has been limited or inconsistent, our study will contribute to a more comprehensive understanding of the effectiveness of these substances.

The use of these substances would also aid in slowing the rampant rise antimicrobial resistance and mitigate its negative effects.

1.4 Study Question

What are the differences in the antimicrobial effect of Manuka honey, local honey, sugar and sugarcane molasses and silkworm sericins on commonly isolated wound microbes?

1.5 Alternative Hypothesis

There are differences in the group means of the antimicrobial effect of Manuka honey, local honey, sugar, sugarcane molasses and silkworm sericins on commonly isolated wound microbes.

1.6 Study Objectives

1.6.1 Broad Objectives

To compare the antimicrobial effect of Manuka honey, local honey, sugar, sugarcane molasses, silkworm sericins, and a control (0.9% saline) on commonly isolated wound microbes.

1.6.2 Specific Objectives

As regards to samples cultured with Manuka honey, local honey, sugar, sugarcane molasses, silkworm sericins and control groups:

a) To determine antimicrobial susceptibility

b) To determine the minimum inhibitory concentration (MIC).

c) To determine the minimum bactericidal concentration (MBC).

d) To compare the MIC and MBC of the different natural antimicrobial substances

e) To compare the MIC and MBC of the different antimicrobial substances with a control

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 Bacterial Invasion and the Impact on Wound Healing

Wounds compromise the integrity of the skin thereby allowing bacteria that are typically sequestered on the skin surface to infiltrate to the underlying tissues at varying extents. The ability of this to occur is often propagated by a number of factors such as bacterial bioburden, local deprivation of arterial supply, poor venous drainage, comorbidities or concomitant wound trauma (R and Kg, 2004). Bacterial interaction with wounds occurs over a spectrum ranging from colonization, local infection, and in some cases, systemic infection which may present as cellulitis or septicemia.

Wounds can be classified based on the infection and replication status of bacteria. Clean wounds are infection-free, resulting from small cuts or sterile surgical incisions. Clean-contaminated wounds occur when aseptic procedures involve body parts with high bacterial counts. Contaminated wounds stem from lapses in sterile technique or traumatic injuries with non-sterile instruments. Dirty or infected wounds are already infected or result from perforation of infected hollow organs, exhibiting pus or abscesses. Bacterial replication status is also considered. Non-replicating bacterial infections occur in poorly oxygenated wounds, while replicating bacterial infections involve actively multiplying bacteria.

Based on the replication status, non-replicating bacterial infections involve bacteria that are not actively replicating and are commonly found in wounds with limited oxygen supply, such as those with anaerobic bacterial infections. Replicating bacterial infections involve bacteria that are actively multiplying and can cause rapid infection spread, including wounds with aerobic bacterial infections.

Wounds are defined as contaminated when non-replicating bacteria are present or colonized when bacteria replicate in the wound without tissue impairment. An intermediate stage (local infection or critical colonization), occurs when bacterial replication triggers local tissue responses and marks the transition between colonization and invasive infection of the wound. On extreme situations, invasive infection ensues, where presence of replicating bacteria within the wound causes host injury (Guo and DiPietro, 2010).

In response to wounds, host tissue responds through a series of coagulation, inflammatory, proliferation and re-epithelialization processes. This occurs with the help of mediators such as cytokines, growth factors or inhibitors, platelets, inflammatory cells, extracellular matrix and proteinases (Garraud et al., 2017). Coagulation and inflammatory phases occur almost instantly after skin damage, with inflammation lasting up to day 6. Proliferation then marks the

commencement of angiogenesis and extracellular matrix development, followed by remodeling which begins about 3 weeks post-injury and lasts up to 2 years.

Prolongation of the aforementioned processes, especially the inflammatory or proliferative stages, often result in impaired healing and subsequent excessive scar tissue (Negut et al., 2018). In this regard, wounds are classified as either acute, when healing occurs through the regular stages, or chronic, when repair and healing takes significantly longer. Healing time is often influenced by several factors such as; amplification of inflammatory mediators, presence of infection, hypoxic states, inadequate nutrition, elderly age, wound dryness or in existence of underlying conditions such as diabetes mellitus (Demidova-Rice et al., 2012). Of note, wound bacterial infection accounts significantly in the delay of the wound healing process by influencing the degree of inflammation.

Chronicity of wounds in the setting of bacterial infection occurs due to prolongation of IL-1 and tumour necrosis factor-α inflammatory cytokine levels. This prolonged elevation leads to increased matrix metalloproteases (MMP), a decrease in MMP inhibitors, and granulation tissue formation factors (Guo and DiPietro, 2010). Sub-infective bacterial levels seem to hasten wound healing and granulation tissue establishment. This is demonstrated through associated increase in neutrophil, monocyte and macrophages infiltration, elevation of prostaglandin E2, and collagen formation. However, infective levels and the bacterial exotoxins attack different cell types, causing tissue necrosis aggravated by local hypoxaemia due to vessel occlusion (R and Kg, 2004).

2.1.1 Commonly Isolated Organisms

According to literature, the most common Gram-positive bacteria implicated in burn wound infections include Staphylococcus spp., Enterococcus spp., and beta-hemolytic Streptococcus group A (GAS). While the most frequently isolated Gram-negative bacteria from patients with burn wounds include Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella spp., and Escherichia coli. Some of the more common fungi that cause wound infections include Candida spp., Fusarium spp., Mucorales (e.g., Rhizopus, Mucor, or Rhizomucor), and Aspergillus spp. *Staphylococcus aureus,* composes the most isolated bacteria in various wounds. *Pseudomonas aeruginosa* on the other hand is commonly observed in surgical and burn wounds, while *Enterococcus* and *Enterobacteriaceae* are seen in immunocompromised individuals and abdominal surgeries. A study in Tanzania observed *S. aureus* in 29.1% of acute wounds, *Pseudomonas aeruginosa* in 18.2% and other Coliforms in 23%. *Proteus mirabilis* was isolated

in 26.9% of chronic wounds, while *Enterococcus* and *Escherichia coli* consisted 23.1% (Kassam et al., 2017).

Bacterial communities may also exist in wounds as a biofilm. They are organized in small colonies encapsulated within an extracellular polymeric matrix. This matrix is separated by a pseudo-circulatory system composed of open water channels, essential for supply of nutrients and waste products removal. The polymeric material, acting as a physical barrier, prevents infiltration and hence impedes the action of antimicrobials (Davies, 2003). Biofilms offer bacteria physical protection and enable their cross-communication and quorum sensing. This leads to increased virulence and proclivity to cause infections.

These biofilms are mostly found in chronic wounds due to the presence of collagen and fibronectin proteins as well as damaged tissues. These factors favor their attachment and further enhance the chronicity of wounds. Pseudomonas, and methicillin-resistant *Staphylococcus aureus* (MRSA), predominantly chronic wound bacteria, are characteristic biofilm formers (Wolcott and Rhoads, 2008). Due to the aforesaid advantages of biofilms, bacteria residing within exhibit extreme antibacterial resistance (up to 1000 times) as compared to freely living equivalents. Further, the slow bacterial growth that occurs in biofilms appears to favor decreased drug uptake and confers bacterial physiological changes that weaken antibiotic effectiveness (Siddiqui and Bernstein, 2010).

2.2 Antibacterial Management of Wounds

Proper care and management of wounds remains paramount to not only promote expedited healing but also in order to prevent concomitant morbidity and mortality secondary to wound infections. With the almost inevitable occurrence of wound bacterial infections and the associated consequences, basic cleaning of wounds and additional use of antibacterial agents therefore remain necessary in order to try achieve a bacterial bioburden that is relatively host-manageable (Negut et al., 2018).

Wound dressings that are moisture retentive, despite their capacity to increase bacterial counts, have over time been employed to reduce the rates of wound infection by maximizing activity of neutrophils and preventing formation of dry necrotic debris and dead space that favors bacterial growth. Occlusive dressings on the other hand are observed to create an anaerobic state which further increase both floral and non-floral bacterial replication. To account for the downside of traditional wound dressings, several therapeutic complexes have been added onto the dressings to allow functionalized delivery of these agents into wounds (R and Kg, 2004). As such, dressings have been impregnated with different antibiotics including cephalosporins,

quinolones, aminoglycosides and tetracyclines among others. Topical antibiotics such as silver, iodine and chlorhexidine may have often been preferred due to their additional effects in wound healing while systemic antibiotics have been mostly reserved for use in patients with widespread infection (Negut et al., 2018; Ramasubbu et al., 2017).

Biofilms, however, still pose a significant challenge in management of bacterial wound infections. They represent a community of microorganisms that adhere to a surface and secrete a sticky substance known as extracellular polymeric substance (EPS) that forms a protective and adhesive matrix around them. Additionally, biofilms can produce toxic substances that can damage the surrounding tissue and exacerbate inflammation, leading to chronic wounds that are difficult to treat. Biofilms can also contribute to antibiotic resistance, as they are often composed of multiple species of microorganisms that can share genes for antibiotic resistance. Presently, removal of the biofilm through regular debridement by means of a sharp or sterile gauze has proven effective. This is coupled with day-to-day use of an antiseptic solution that is non-toxic. In addition to direct reduction of bioburden and bacterial toxins, debridement also facilitates clearance of devitalized tissue and any debris, thus cutting down bacterial nutrient supply (Siddiqui and Bernstein, 2010).

The main challenge currently in the fight against bacterial wound infections is the emergence and increase in antibiotic-resistant bacteria, especially due to misuse or overuse of these agents. This crisis has greatly impaired the effectiveness of antibiotic-impregnated wound dressings especially in long term treatment of chronic wounds such as diabetic foot and pressure ulcers (Negut et al., 2018). This has further been worsened by the lack of creation of novel antibiotic agents that would otherwise overcome the challenge with antimicrobial resistance. As a result, there has recently been renewed interest in the use of unconventional non-antibiotic agents and especially natural agents such as honey, sugar, molasses and sericins (Mphande et al., 2007; Rahiman and Pool, 2016; Scagnelli, 2016, Aramwit 2013). Further, with regards to chronic wounds, wherein long-term treatment requires regular changing of the wound dressing, most of these natural agents offer options that are completely dissolvable, non-adherent and do not require replacement (Negut et al., 2018).

2.3 Honey as an Antibacterial Wound Dressing

Since ancient times, honey has widely been used clinically due to its antimicrobial activity and positive influence on wound healing. It has been demonstrated to have concentration-dependent bacteriostatic and bactericidal effects by van Ketel in 1892 (Petreanu, 1979). Its effect has been demonstrated on about 60 bacterial species including anaerobes, aerobes, gram

positive and gram-negative bacteria. Its antimicrobial effect has been seen in vivo on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* which are commonly encountered on wounds (Chambers, 2006; Maeda et al., 2008; Natarajan et al., 2001).

Honey's antibacterial effect is attributed to a couple of elements such as its hydrogen peroxide constituent, high sugar content which causes bacterial dehydration and a low pH (3.2-4.5). Several phytochemical agents, glucose oxidase, catalase and chemical factors such as beeswax, propolis, nectar, pollen lysozymes and organic acids have also been attributed to its antibacterial properties (Basualdo et al., 2007; Mandal and Mandal, 2011). Honey also has capacity to stimulate monocytes to secrete TNF-α, known for its potential to induce wound repair. Furthermore, honey reduces release of reactive intermediates hence limiting tissue damage that can be caused by activated macrophages (Tonks et al., 2001).

Besides, its effect on antibiotic resistant bacteria, its speedy rate of infection clearance, fast debridement of wounds and rapid quelling of inflammation, have increased its uptake in wound care (Basualdo et al., 2007). It also has the additional benefits of reduction of scar formation, stimulation of angiogenesis as well as tissue granulation and epithelium growth that have further favored its adoption in wound dressing (Tonks et al., 2001 and Natarajan et al., 2001).

2.3.1 Antimicrobial Effects of Manuka Honey

In 1981, it was discovered that honey from flowers of the native Manuka trees of New Zealand (Manuka honey) had extraordinarily superior antiseptic properties. Though similar to 'regular' honey in its composition, it was observed to contain inordinately high levels of methylglyoxal (MGO) resulting from dehydration of its antecedent phytochemical dihydroxyacetone (DHA). The former compound has demonstrated selective toxicity where it spares mammalian cells but is toxic to bacterial cells (Adams et al., 2009). Other contributing factors to its antibacterial property are its high osmolality, osmotic pressure, low pH and protein content, flavonoids, phenolics, and a high carbon: nitrogen ratio. These factors are especially important since they give manuka honey the capacity to still display substantial antibacterial activity even in cases where hydrogen peroxide activity is inhibited (Mandal and Mandal, 2011). The potency of Manuka honey is rated using a system known as Unique Manuka Factor (UMF) which illustrates the equivalent mass concentration of phenol (%w/v) that is needed to produce similar antibacterial activity as honey. There is a direct correlation between the UMF rating of Manuka honey, its levels of MGO and its antibacterial action (Adams et al., 2009).

Honey has, therefore, gained vast acceptance for its use in treatment of bed sores, ulcers, as well as other skin infections that may result from burns and other wounds. The therapy of diabetic foot ulcers is one instance of how honey is used in wound care. Manuka honey is a good example of medical-grade honey that is chosen for its recognized antibacterial action (Alam et al., 2014). When used topically, its speedily clears infection on wounds hence facilitating rapid healing of deeply infected surgical wounds (Mandal and Mandal, 2011). Furthermore, its use has also been successfully demonstrated on skin grafts as well as in graft donor sites that are infected. Clinically, reduced inflammatory symptoms have been observed following honey application on wounds especially due to its role in removal of inflammatory exudates (Ahmed et al., 2003).

2.3.2 Antimicrobial Effects of Local Honey

Several studies have shown that local Kenyan honey has antimicrobial activity as it contains bioactive contents, bio-functional properties in the range or higher than other honey reported in the literature (Mokaya et al., 2019). Though it has lower antimicrobial activity than Manuka and Cuban honey, local Kenyan honey also displays marked antibacterial activity and marked disruption of preformed biofilms (Morroni et al., 2018). It is thought to bring about these antibacterial effects through hydrogen peroxide generation, its phytochemical constituents, radical scavenging and non-peroxide antimicrobial activities (Mokaya et al., 2019).

Local honey was also noted to be active against a wide range of microbes including *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans* (Muli et al., 2008; Morroni et al., 2018), most of which are commonly encountered wound microbes. Therefore, locally available honey may be applied in the treatment of bed sores, ulcers, as well as other skin infections that may result from burns and other wounds. Topical use will speed infection clearance from wounds hence facilitating rapid healing of deeply infected surgical wounds (Mandal and Mandal, 2011).

2.3.3 Sugar as an Antibacterial Wound Dressing

The use of sugar in wounds dates back to pre-modern times in early Mesopotamia. In 1679, Johannes Scultetus documented the use of fine powder sugar in cleaning of wounds. Zoinin, later in 1714, further emphasized the role of sugar in the promotion of wound as well as ulcer healing. In recent times, use of sugar in wound healing has gained vast attention in light of antimicrobial resistance as well as its availability and affordability especially in developing

countries (Biswas et al., 2010). While some literature suggests that use of sugar in wounds may cause systemic hyperglycemia and ultimately impair host defenses mechanisms, counter-evidence shows that both local and systemic hyperglycemia do not enhance impairment of wound healing in isolation (Bagdade et al., 1978; Trousdale et al., 2009).

Instead, direct application of sugar on a wound wields local osmosis hence promoting formation of granulation tissue, reducing edema and lowering wound pH which increases its bacteriostatic capacity. Direct application also results in promotion of small vessel dilation, bacterial lysis, and inhibiting bacterial growth through lowering the water activity critical for growth to occur. Other advantages of using sugar in wounds include its ability to nonspecifically destroy bacteria, drawing macrophages to the wound and propagation of wound cleansing (Biswas et al., 2010; Chirife, 1982)

Sugar has been employed in treatment of a variety of wounds inclusive of surgical site wounds, burn wounds as well as in diabetic ulcers (Biswas et al., 2010). Its antibacterial properties have further been successfully demonstrated in *Staphylococcus aureus*, MRSA *and Pseudomonas aeruginosa*, as some of the most common bacteria causing wound infections (Biswas et al., 2010). It also has minimal associated systemic effects since sucrose is itself not metabolized extra-intestinally, however, its use in large open wounds was reported to cause acute renal failure and hyponatremia (Debure et al., 1987).

2.3.4 Sugarcane Molasses as an Antibacterial Wound Dressing

Sugarcane molasses is a dense, dark substance that is the final effluent of sugar refinement and has high mineral content. It comprises of approximately 46% sugars, other organic materials such as phenolics and other compounds such as melanoidins produced during the manufacturing process. Traditionally, it has gained use as an alternative sweetener in several foods (Rahiman and Pool, 2016). Globally, its used in enhancing digestive microbial growth in livestock hence promoting fibre and non-protein nitrogen digestion.

Its therapeutic use is based on the basis of its rich mineral content (Wang et al., 2011). Little scientific evidence however exists documenting its health-related benefits. Nagai and Koge reported physiological effects of various sugarcane extracts. They suggested that these extracts increase defense against both bacterial and viral pathogens; enhancing immune responses and also possessing potential hepatoprotective and antioxidant characteristics (Nagai et al., 2001). Guimarães, further emphasized that sugarcane elements that exhibit the aforementioned characteristics are found in molasses (Guimarães et al., 2007).

A study evaluating effects of sugarcane molasses and other compounds on wound desiccation, and wound healing features of *Staphylococcus aureus* biofilm on a rabbit ear showed development of a dry scab, which led to displacement of majority of bacteria within the biofilm from the wound bed. Further, the wounds expressed significantly lower levels of TNF- α and IL-1b inflammatory markers and showed increased formation of granulation tissue. Additionally, *Staphylococcus aureus* growth in vitro was inhibited suggesting potential use in reducing bacterial bioburden as well as inflammation while increasing formation of granulation tissue (Park et al., 2016).

2.3.5 Silkworm Sericin in Wound Healing

The silkworm, *Bombyx mori*, cocoon is made up of two proteins, sericin and fibroin. *Bombyx mori* is an insect that belongs to the moth family *Bombycidae* and order *Lepidoptera*. *B. mori* is one of the most important lepidopterans used for scientific research. Fibroin is the insoluble fibrous protein component of the cocoon. Sericin is the hydrophilic glycoprotein that serves to bind fibroin strands in order to form the cocoon (Soumya, M. et. al 2017). It comprises 25% to 30% of the total weight of the cocoon (Kundu, R. et. al 2016). B. mori cocoon has been the subject of major research and its shown benefit in use in the biomaterial and polymers sectors and the cosmetics and food industries at large (Joseph B. et. Al 2012), (Padol A. et.al. 2012).

The cocoon components are separated by the process of unspinning and degumming. Fibroin is processed into raw silk which is commercially used in fabric production. Sericin, which used to be discarded in wastewater, has recently found its use in non-textile applications. And this has been fueled by the growing need of biocompatible and biodegradable compounds. Silk has had extensive medical applications since ancient times (Holland, C. et. al. 2019) and silk proteins are touted as the emerging frontier of biopolymers that promise utility in wound healing and regeneration.

Sericin molecules physicochemical characteristics are responsible for its biomedical uses. They are shown to have antimicrobial, antitumor, antioxidant, anticoagulant properties with good oxygen permeability and moisture organizing qualities (Kurioka et al. 2004; Zhang 2002). Sericin has been shown to be immunologically inert therefore making it a safe biomaterial of choice and opening wide its potential application in the field of biomedicine. It has also been introduced to cell lines in various culture media and has not shown any cytotoxicity (Terada, S. et. al. 2002).

Aramwit et al. in 2009 evaluated the inflammatory mediators induced by sericin both in vitro and in vivo set ups. In the in vivo studies, they discovered that there was a decrease in expression of TNF-α and IL-1β in the tissues and generally, the entire wound healing process

was expedited. Through this study, it was evident that sericin favored wound healing without worsening the inflammatory process.

Another study recently published showed that Silk Sericin extracted through heat, acid, alkali, or urea, showed ability to exhibit antibiofilm activity in wounds (Aramwit, P. et. al. 2020). In yet another study by Aramwit et al., they applied a standard antibiotic cream of silver sulfadiazine mixed with an 8% preparation of sericin for the treatment of open wounds caused by second-degree burns. A blind evaluation evidenced that sericin expedited wounds closure. The mean duration required to achieve 70% of epithelialization of the burn surface to full and complete healing in the treated cohort was markedly shorter than the control group (without sericin) by about 5–7 days. A decrease in average length of hospitalization and patients' pain, improving their quality of life was also demonstrated. This information has advised our study on the ability of sericins to possess antimicrobial activity.

The literature review highlights several natural products that have been used as antibacterial wound dressings, including honey, sugar, sugarcane molasses, and silkworm sericin. Honey, especially Manuka honey, has been extensively studied and found to have potent antimicrobial properties attributed to various factors such as its hydrogen peroxide constituent, high sugar content, and low pH. Sugar, on the other hand, has been used for centuries and has been found to have local osmosis, bacteriostatic, and bactericidal effects, making it effective in treating a variety of wounds. Sugarcane molasses has been shown to have potential antimicrobial and anti-inflammatory properties, as well as the ability to increase granulation tissue formation. Silkworm sericin, a hydrophilic glycoprotein, has been found to have various physiological and immunological properties, including antimicrobial, antioxidant, antitumor, and anticoagulant activities, making it a promising biomolecule for wound healing and regeneration.

The use of natural products in wound healing has gained significant attention in the medical field, particularly due to the emergence of antimicrobial resistance and the need for cost-effective and readily available wound dressings. These natural products offer several advantages such as their effectiveness against a broad spectrum of bacteria, low toxicity, and ability to promote wound healing. However, more research is needed to fully understand their mechanism of action and optimal use in wound care.

2.4 Conceptual Framework

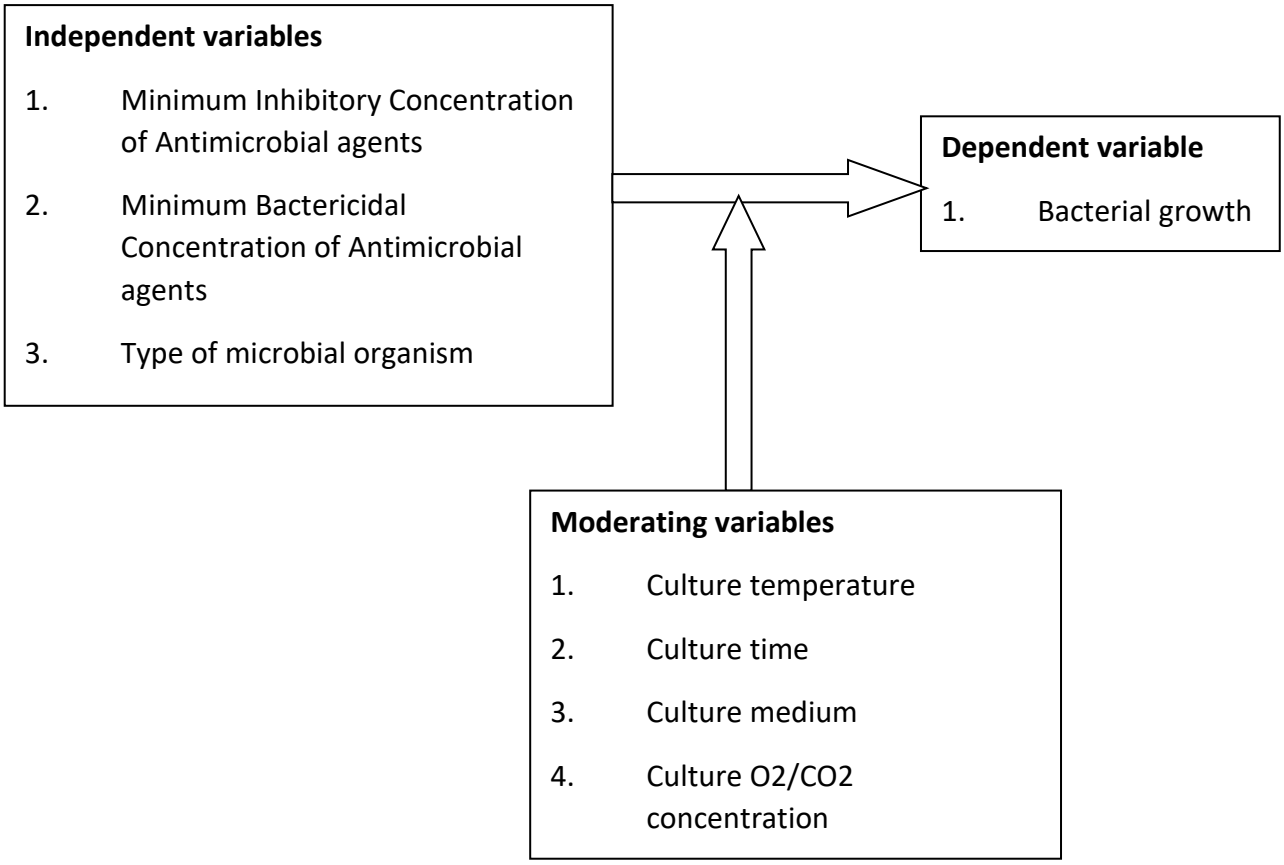

Figure 1:Conceptual framework

3.0 CHAPTER THREE: METHODOLOGY

3.1 Study Design

Controlled quasi-experimental laboratory study

3.2 Study Area and Site Description

This study was conducted at Kenyatta National Hospital (KNH) and the University of Nairobi, Department of Microbiology. KNH is the largest referral hospital in Kenya. It is a public, tertiary, referral hospital and serves as a teaching hospital for the Faculty of Health Sciences of The University of Nairobi. It has a capacity of 1,800 beds and patient numbers can rise to as high as 3,000.

Kenyatta National Hospital has a Plastic, Reconstructive and Aesthetic Surgery Unit that manages amongst other conditions, burns patients, chronic wounds, and bed sores.

The study was also carried out at the Department of Microbiology, University of Nairobi, which was the unit where culture and analysis of samples was conducted. The department covers medical parasitology, bacteriology, mycology, medical virology, entomology, molecular biology and immunology. It includes teaching and research laboratories, clinic rooms, seminar rooms data rooms, administrative offices and collaboration facilities with UNITID and KAVI-ICR.

3.3 Selection Criteria

3.3.1 Inclusion Criteria

a) Monofloral Manuka honey obtained from Healthy U -The Hub Karen.
b) Local honey obtained from the Kenya National Beekeeping institute in Lenana, Nairobi.
c) Processed silk sericin obtained from Silk Origin Kenya Limited
d) Blackstrap sugarcane molasses were purchased from local suppliers
e) Mumias brown sugar was purchased from Naivas supermarkets, a local supermarket chain in Kenya.

These antimicrobial substances were also chosen due to their easy availability and acquisition. Each antimicrobial test sample, i.e., sugar, honey and molasses, used for the study was consistently obtained from the same supplier (preferably a collector who sources the products from the same farm) to maintain controlled experimental quality of the substances hence minimizing any potential confounders to the outcomes of interest. The Hub Karen retail stores

is one of the most accessible stores that stock Monofloral Manuka Honey in the city of Nairobi. Local honey from Meru was the honey that was available at the Kenya National Beekeeping institute in Lenana, Nairobi, at the time of sample collection. It was selected due to the convenience of availability. This Meru sourced local honey was supplied to the institutes laboratory directly by the farmer and was subjected to laboratory testing to ensure it met all criteria for unadulterated honey as indicated further below. Mumias brown sugar was selected as it is a common Kenyan sugar brand available in retail shops and supermarkets. Blackstrap molasses were also selected at random from brands available at local supermarkets.

3.3.2 Exclusion Criteria

Antimicrobial samples obtained from other regions apart from the ones listed above were excluded. Multifloral Manuka honey was also excluded. Local honey from other regions and suppliers not mentioned above was excluded. Local honey not subjected to laboratory testing to determine its unadulterated nature was also excluded. Other sugar brands apart from Mumias sugar were excluded.

3.4 Sample Size Determination

Open-Epi website version 3.01 updated 4th June 2013 (Dean AG et al., 2013) was used to calculate the number of microbes required for the study. Microorganism sample size calculation for an analytical cross-sectional study was done as outlined below yielding a size of 24 per antimicrobial test sample group. The reference percent of exposed with outcome (34.84%) was obtained from a study assessing the effects of honey and sugar dressings wound healing (Mphande et al., 2007). Power was set at 80%. The photos below show the website's page and how the sample size was calculated.

Clear Calculate

Sample Size:X-Sectional, Cohort, & Randomized Clinical Trials		
Two-sided confidence level(%)	95	(1-alpha) usually 95%
Power (1-beta or % chance of detecting)	80	Usually 80%
Ratio of Unexposed to Exposed in sample	1.0	For equal samples, use 1.0
Percent of Unexposed with Outcome	5	Between 0.0 and 99.9
Please fill in 1 of the following. The others will be calculated.		
Odds ratio	12.15	
Percent of Exposed with Outcome	39.01	Between 0.0 and 99.9
Risk/Prevalence Ratio	7.80	
Risk/Prevalence difference	34.01	Between -99.99 and 99.99

Sample Size:X-Sectional, Cohort, & Randomized Clinical Trials

Two-sided significance level(1-alpha):	95
Power(1-beta, % chance of detecting):	80
Ratio of sample size, Unexposed/Exposed:	1
Percent of Unexposed with Outcome:	5
Percent of Exposed with Outcome:	39
Odds Ratio:	12
Risk/Prevalence Ratio:	7.8
Risk/Prevalence difference:	34

	Kelsey	Fleiss	Fleiss with CC
Sample Size - Exposed	24	23	28
Sample Size-Nonexposed	24	23	28
Total sample size:	48	46	56

References

Kelsey et al., Methods in Observational Epidemiology 2nd Edition, Table 12-15
Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 &3.19
CC = continuity correction
Results are rounded up to the nearest integer.
Print from the browser menu or select, copy, and paste to other programs.

Results from OpenEpi, Version 3, open source calculator--SSCohort
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

N= 24 isolates per microorganism, for 6 groups (Manuka honey, local honey, molasses, sugar, silkworm sericins and control). However, to account for 8% attrition rate, 26 isolates per microorganism were used.

3.5 Study Variables

3.5.1 Dependent Variable

Antimicrobial activity, MIC, MBC

3.5.2 Independent variables

Number of positive culture plates.

3.6 Microbiology experiments

3.6.1 Sample Acquisition

Manuka honey was obtained from local retail stores in Nairobi (Healthy U-The Hub, Karen). The batch number assigned to this lot was 213278. The certificate of analysis to show that this was tested and certified as monofloral Manuka honey is attached in the appendix IV.

Figure 2:Photos of the certified Manuka honey

Local Honey from Meru, was obtained from the Kenya National Beekeeping institute in Lenana, Nairobi. This honey was tested at a laboratory under the Institute, to make sure it met

the requirements to pass as genuine unadulterated honey. The tests done assessed moisture content of the honey, total reducing sugars, sucrose levels, pH and hydroxymethylfurfural concentration. This laboratory analysis showed that the honey was not adulterated. The honey used for this research was honey from Meru collected on the 15th March 2023. The certificate of analysis is attached as appendix V.

Figure 3:Photo of certified Local honey

Blackstrap sugarcane molasses were purchased from local suppliers. We were unable to test the composition of the sugar molasses nor sugar.

Figure 4:Photo of Molasses

Brown sugar was purchased from local supermarkets.

Processed silk sericin was provided by Silk Origin Kenya Limited. The batch used for this research was at a concentration of 2.1% (w/v%).

Figure 5:Photo of a cocoon that is processed to obtain silk sericins

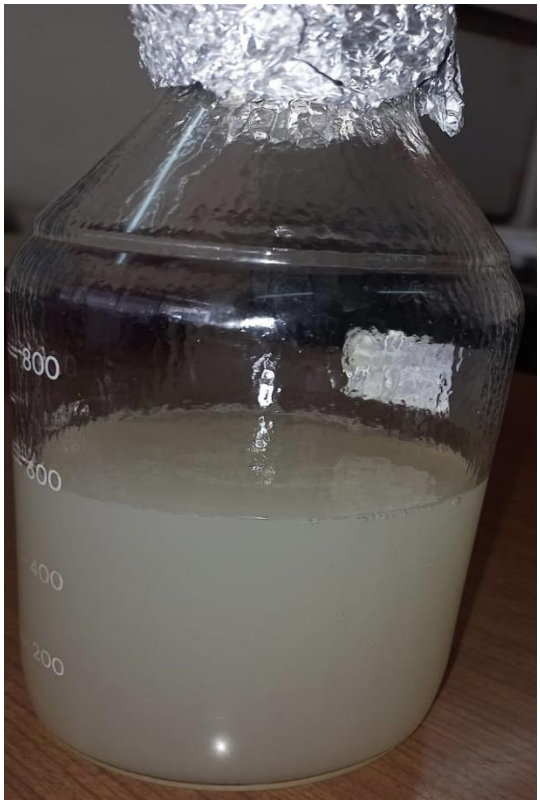

Figure 6:Photo of Silk Sericin

3.6.2 Sample Preparation

Standard culture bottles with 2.5 mL of soy tryptone broth (Oxoid Limited; Thermo Fischer Scientific) each labelled and serialized were added each into them 2.5mls of Manuka honey, 2.5mls local honey, 2.5g sugar, 2.5 mls molasses, 2.5mls silkworm sericin or 2.5mls normal saline as the negative control to prepare 50% (w/v%) of the test samples. Serial dilutions were then done by adding 2.5mls of this prepared 50% (w/v%) to 2.5mls of the soy tryptone broth. This was done to achieve further dilutions of 25%, 12.5%, and 6.25%.

3.6.3 Specimen Collection and Acquisition

Specimen included microbes (bacteria and fungi) commonly isolated from either open or infected burn wounds, chronic wounds or surgical site infections. According to literature, the most common Gram-positive bacteria implicated in burn wound infections include Staphylococcus spp., Enterococcus spp., and beta-hemolytic Streptococcus group A (GAS). While the most frequently isolated Gram-negative bacteria from patients with burn wounds include Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella spp., and Escherichia coli. Some of the more common fungi that cause wound infections include Candida spp., Fusarium spp., Mucorales (e.g., Rhizopus, Mucor, or Rhizomucor), and Aspergillus spp.

Table 1:Readily available Isolates

Type of Microbe	Source of isolation	Microorganism
Gram positive bacteria	Open or infected burn wounds, chronic wounds, surgical site infections	Staphylococcus Aureus
Gram negative bacteria	Open or infected burn wounds, chronic wounds, surgical site infections	Pseudomonas Aeruginosa
Fungi	Open or infected burn wounds, chronic wounds, surgical site infections	Candida spp.

The table above showed samples that were readily available as isolates from both the Department of Medical Microbiology labs of the University of Nairobi and Kenyatta National Hospital.

3.6.4 Microbiology Culture Analysis

Microorganisms already cultured and isolated were acquired from the Microbiology department, Kenyatta National Hospital and University of Nairobi Microbiology department. These isolates were collected from the petri dishes using sterile swabs and inoculated into sterile cryovials containing autoclaved skim milk. The cryovials were labelled in accordance with the serial numbers assigned to the microbes. These cryovials were then frozen at -40 degrees Celsius and were thawed for microorganism retrieval on a need basis. Furthermore, the microbiology department provided culture media (nutrient agar) and tryptone soy broth (Oxoid Limited, Thermo Fischer Scientific). All microorganism handling procedures were carried out in a class 2 biological safety chamber. The microbes were inoculated into sterile cryovials containing sterilized skim milk that were marked and serialized with the type of microbe in them. They were then frozen at -40 degrees Celsius for revival during the course of the research.

The isolates were then revived/pure cultured by streaking them on nutrient agar petri dishes for the bacteria and Sabouraud dextrose agar for the candida spp. to get one specific isolate for microbial culture in the research. Both nutrient agar plates and Sabouraud agar plates were incubated at 37 degrees Celsius for 24 hours and 30 degrees Celsius for 48 hours respectively. Sabouraud agar plates on the other hand were incubated in aerobic conditions at 30° C for a period of 48 hours. After the incubation period and on confirmation that there was growth on

the culture media, the dishes were retrieved from the incubator. 0.5 McFarland standard were prepared for each of the isolates of the microorganisms under study. 100 microliters of the pure culture McFarland standard solutions were then transferred to a set of standard culture bottles labelled and serialized containing 2.5g 100% Manuka honey, 2.5g 100% local honey, 2.5g 100% sugar, 2.5g 100% molasses, 2.5ml 100% silkworm sericin and 2.5ml of saline in plain broth as the control.

This mixture of microorganisms and the test samples were then incubated under aerobic conditions for at least 16hrs and no more than 24 hours for the bacteria and 48 hours for the fungi. They were then streaked onto labelled petri dishes with nutrient agar. These petri dishes were then incubated again for 16-24 hours for the bacteria and 48 hours for the fungi. The petri dishes were then retrieved from the incubator and readings recorded into the data collection sheets. The readings were interpreted as no growth and presence of growth. Presence of growth was categorized as one, two, three or four colony forming units, less dense growth for many sparsely populated colony forming units and growth for any population of growth more than the less dense categorization. The petri dishes were then autoclaved to sterilize them in preparation for disposal observing the biohazardous waste disposal protocols laid out in the department.

The soy tryptone broth was prepared by adding 30g of the powder to 1 litre of water (purified as required), mixed well, decanted into a 1 litre glass jar and sterilized by autoclaving at 121°C for 15 minutes. Standard culture bottles with 2.5 mL of soy tryptone broth (Oxoid Limited; Thermo Fischer Scientific) each labelled and serialized were added each into them 2.5mls of Manuka honey, 2.5mls local honey, 2.5g sugar, 2.5 mls molasses, 2.5mls silkworm sericin or 2.5mls normal saline as the negative control to prepare 50% (w/v%) of the test samples. Serial dilutions were then done by adding 2.5mls of this prepared 50% (w/v%) to 2.5mls of the soy tryptone broth. This was done to achieve further dilutions of 25%, 12.5%, and 6.25%. To these mixtures, 0.5 McFarland standard of the microbes were pipetted into each of these standard bottles and their various dilutions. All subcultures were then incubated at 37°C to mimic human body temperatures. The duration was 16-24 hours for the bacteria and 48 hours for the fungi. Each of these subcultures were then streaked onto labelled nutrient agar plates for the bacteria and labelled Sabouraud dextrose agar for the candida species. These plates were then incubated again for 16-24 hours for the bacteria and 48 hours for the fungi.

The minimum bactericidal concentration was determined by reading and recording the value of the highest dilution of the antimicrobial agent/test sample that results in no growth of the

microorganism on the plate. The nimum inhibitory concentration was recorded as the dilution concentration higher than the concentration required to show MBC.

All findings were recorded on the data collection sheet as outlined in the appendix.

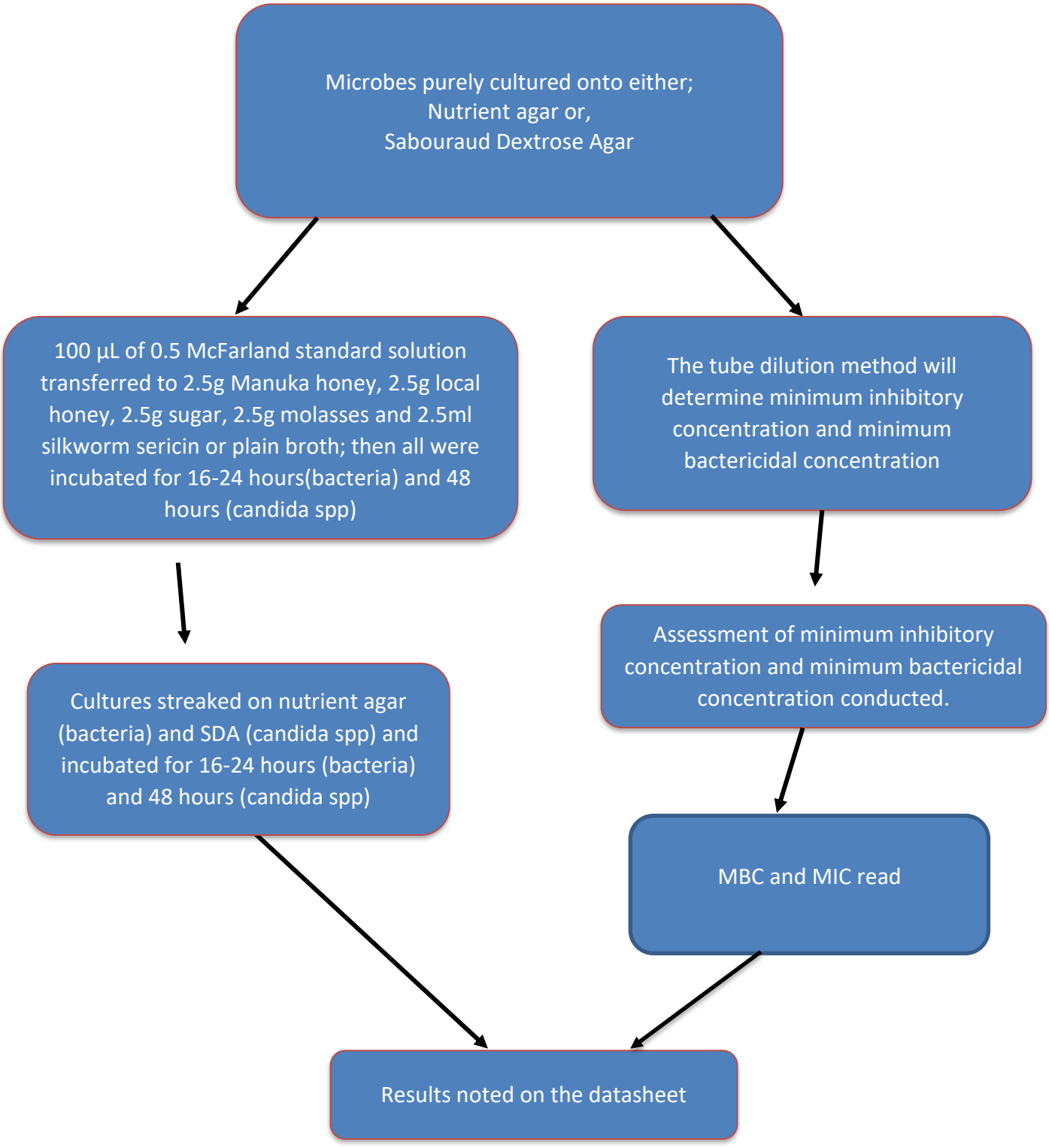

Figure 7:Flow chart illustrating methodology

3.7 Data Management and Results Dissemination Plan

Data collected was entered into IBM SPSS Statistics software (Version 25, IBM Corp.) for cleaning, data encoding and subsequent analysis. Normality of data was evaluated using visual inspection of the Q-Q plots. Descriptive data analysis was reported as means with standard deviations for numerical data. One way analysis of variance (ANOVA) was used to assess for any differences in bacterial growth rate between the 6 groups (manuka honey, local honey, sugar, molasses, sericins and controls), while differences in type of growth rate and presence of bacterial growth was assessed using Kruskal – Wallis test. A p value ≤ 0.05 was considered statistically significant at a 95% confidence interval.

Results obtained were presented in charts, graphs, tables and representative images. Findings from this study was presented in conferences, with manuscripts submitted for peer review in a journal of interest for publication consideration. This shall also be presented at departmental level and submitted as a thesis in fulfillment of the requirements for Master of Medicine in Plastic, Reconstructive and Aesthetic Surgery.

3.8 Quality and Safety Assurance Procedures

Standard specimens obtained from already cultured and isolated microorganisms were used in the study. From the bank of cultured specimens obtained from wound infections, each included specimen selected randomly by species of commonly encountered wound microbes to represent gram positive, gram negative and fungi. Each microbial species included were initially cultured by standard recommended species-specific media and conditions. The experimental procedures for testing antimicrobial properties of the test substances however were the same and carried out under similar controlled conditions. The experimental groups were also compared with negative controls (saline in plain broth) under similar conditions.

Data collection sheets, illustrative images and other study entry files were saved in an external hard disk with system generated password restriction as well as backed up on secure emails and drive folders. The data was initially by cleaned in Microsoft Excel, a process that entailed manually screening and the running of key analytical tests to identify any invalid entries, duplicates, outliers, and other unforeseen inconsistencies that could develop. This analysis was done by visualizing the data in histograms and box plots to identify the pattern of the data and to note data points that deviated significantly from the overall trend.

All study personnel strictly adhered to the Ministry of Health COVID-19 guidelines and measures to minimize contraction of that infection in the course of the study. Within the study areas and laboratory setting, proper infection prevention and control measures such as: donning

of personal protective equipment, maintaining social distance, hand cleaning using soap and enough water for an appropriate amount of time, as well as the use of sanitizers with recommended alcohol levels were observed. Moreover, laboratory equipment was sterilized using autoclaving for any reusable glassware, jik solution for non-autoclavable materials and 70% alcohol for benches and table tops/counters. Proper safety guidelines were also followed to minimize laboratory accidents and spread of the virulent organisms to the laboratory assistants.

3.9 Ethical Considerations

Ethical approval for conducting the study was approved from the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH/UON/ERC) under approval number P732/09/2022 as shown in appendix VI. Unauthorized personnel not involved in data collection or laboratory analysis were barred from having access to any collected data or research material. To ensure this, data collection sheets were shredded soon after entry into the relevant software (Microsoft excel and SPSS) by the statistician. The dataset in the software was encrypted using system generated passwords accessible only to the investigators of the study.

3.10 Study Limitations and Delimitations

3.10.1. Study Limitations

The various study samples (Manuka and local honey, sugar, molasses and silk sericins) were not sterilized for examples by means of gamma radiation due to lack of access and the prohibitive cost of gamma irradiation.

Sugar, sugarcane molasses and honey may differ in the quantity of its constituents based on the region, climate, and soil or nectar profile of the geographical origin.

Silkworm Sericins quality and concentration was limited to what was provided by the local suppliers i.e. Silk Origins Kenya Limited.

3.10.2. Study Delimitations

Despite lack of sterilization of the test samples, only colonies that fit the physicochemical properties of the microorganism in question were scooped for streaking and read during final interpretation of the growths on the petri dishes. Each sample, i.e., sugar, honey and molasses, used for the study was consistently obtained from the same supplier to maintain controlled experimental quality of the substances hence minimizing any potential confounders to the outcomes of interest.

4.0 CHAPTER FOUR: RESULTS

4.1 Normality Testing

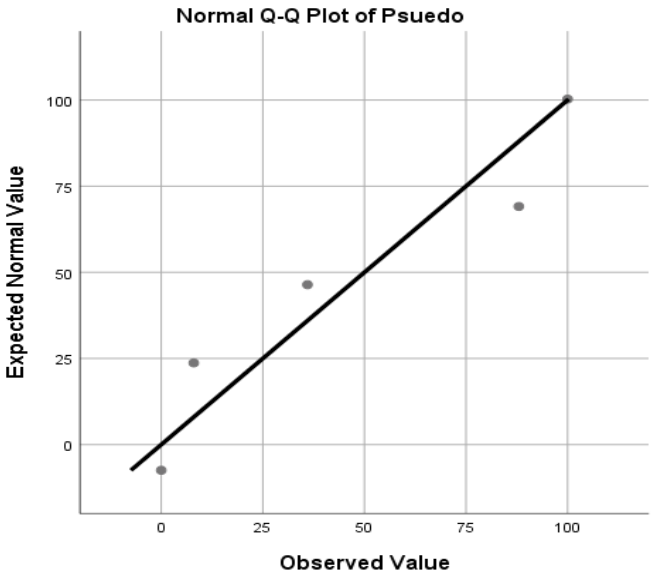

Figure 8:Q-Q plots of pseudomonas aeruginosa

From the figure 8, the plots are far apart. This implies that the data is skewed as the data plots are not close to the line drawn

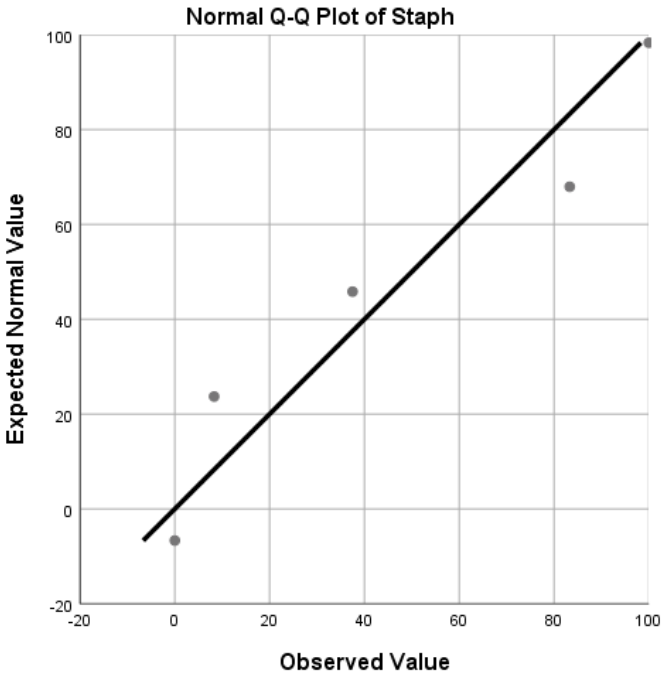

Figure 9:Q-Q plot of Staphylococcus aureus

From the figure 9, the plots are far apart. This implies that the data is skewed as the data plots are not close to the line drawn

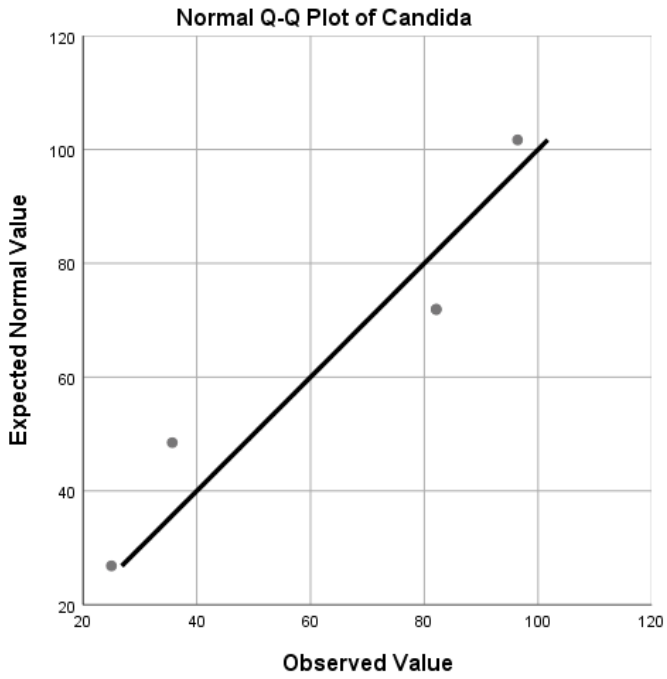

Figure 10:Q-Q Plots of Candida albicans

From the figure10, the plots are far apart. This implies that the data is skewed as the data plots are not close to the line drawn

4.2 Antimicrobial susceptibility

Manuka honey completely inhibited the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in all 25 and 24 samples respectively, as no growth was recorded in these subcultures. However, there was growth observed in 1 of the 28 sub cultured samples *Candida spp* which represented 3.6% of the samples. Table 2 and figure 11 below summarize the antimicrobial susceptibility of the different organisms in Manuka honey.

Table 2:Positive growth patterns in Manuka Honey

Organism	Absolute count / Total samples	Percentage (%)
Pseudomonas	0/24	0
Staphylococcus	0/24	0
Candida	1/28	3.6

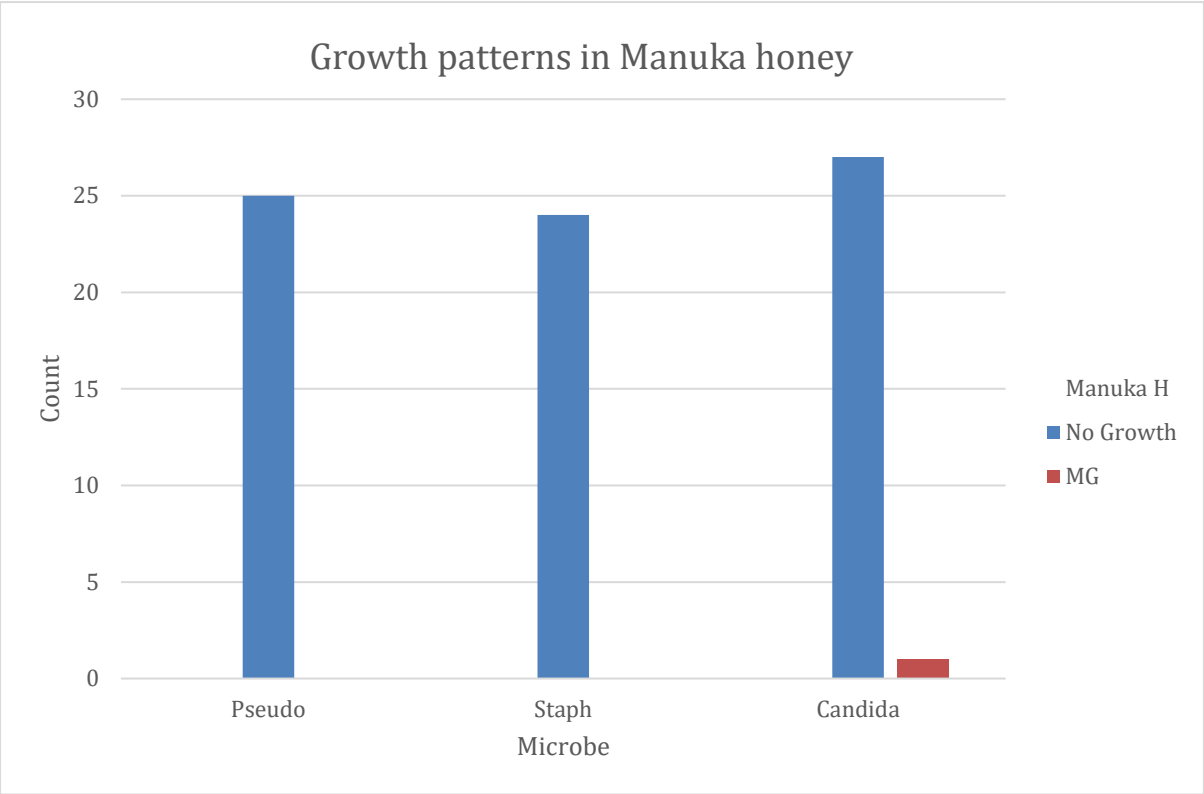

Figure 11:Growth patterns in Manuka honey

Blue bar – absolute count of microbes that showed no growth

Maroon bar – absolute count of microbes that showed moderate growth

MG: Moderate Growth

Percentage growth of *Pseudomonas* in Local honey was at 12% with a sample size of 3 out 25 isolates exhibiting growth, while that of *Staphylococcus* was at 16.7% with growth of 4/24 samples . *Candida spp* was less susceptible to Local honey compared to the bacteria as 17.9% (5 of 28 of the isolates) demonstrated colonial growth of Candida spp as shown below.

Table 3:Positive growth patterns in Local honey

Organism	Absolute count / Total samples	Percentage (%)
Pseudomonas	3/25	12
Staphylococcus	4/24	16.7
Candida	5/28	17.9

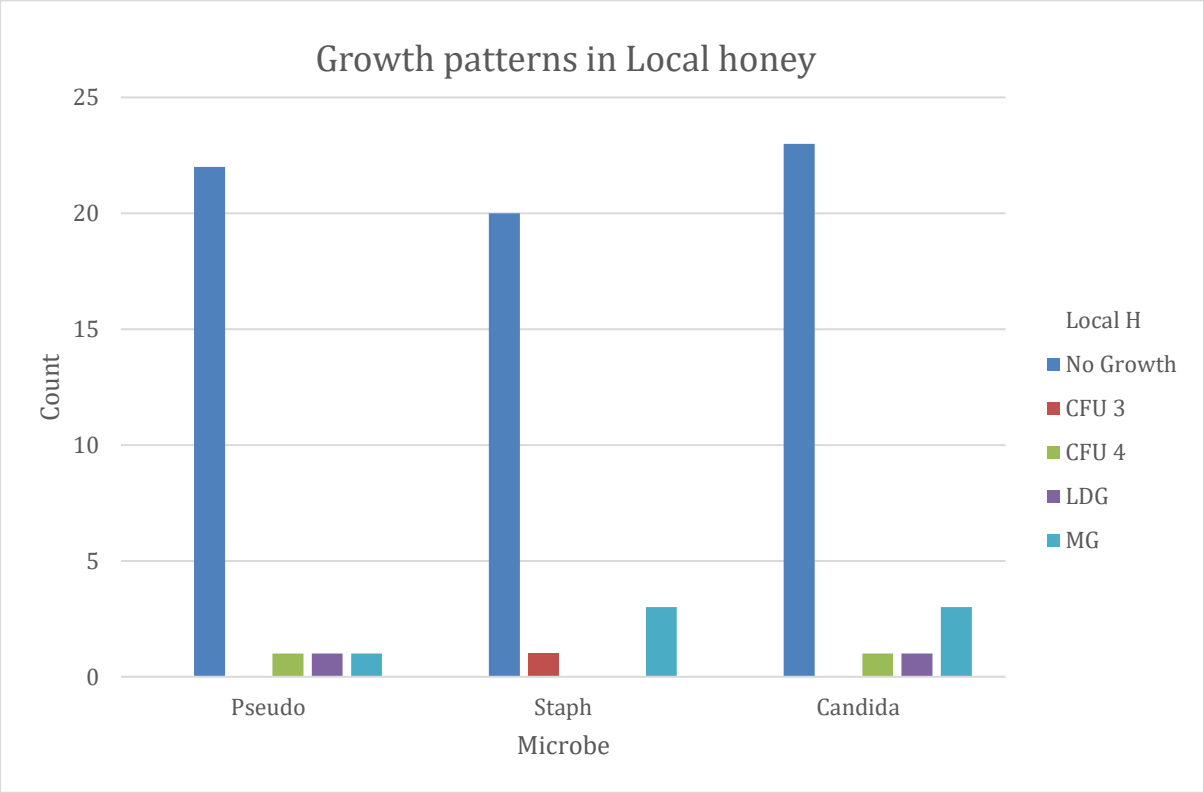

Figure 12:Growth patterns in Local honey

CFU: Colony forming units, LDG: Less Dense Growth, MG: Moderate Growth

Blue bar – absolute count of microbes that showed no growth

Maroon bar – absolute count of microbes that showed CFU 3

Green bar – absolute count of CFU 4

Purple bar - absolute count of LDG

Sky blue bar - absolute count of moderate Growth

Variable growths were observed with Silk Sericin among the organisms. There was 92% (23 out of 25) growth of the 25 samples of *Pseudomonas* recorded. There was no marked difference in growth seen in *Staphylococcus aureus* in Silk Sericin compared to *Pseudomonas* as almost a similar number of colonies of *Staphylococcus* and *Pseudomonas* were observed with 22 of the 24 samples showing growth. Representing 91.7% growth. On the other hand, 75% of the 28 *Candida spp* samples had presence of growth as demonstrated in the table and graph below. Less dense growth refers to a microbial culture or population that exhibits a lower density or abundance of cells, indicating a relatively small or sparse number of microorganisms in the sample or culture. On the other hand, moderate growth describes a microbial culture or

population with an intermediate density of cells, indicating a moderate or intermediate number of microorganisms present. Colony-forming units (CFUs) are used to estimate the number of viable microorganisms in a sample. CFUs represent either a single viable microorganism or a cluster of cells originating from a single viable microorganism. CFUs are commonly used as a unit of measurement in microbiology to quantify the number of viable microorganisms present.

Table 4:Positive growth patterns in Sericin

Organism	Absolute count / Total samples	Percentage (%)
Pseudomonas	23/25	92
Staphylococcus	22/24	91.7
Candida	21/28	75

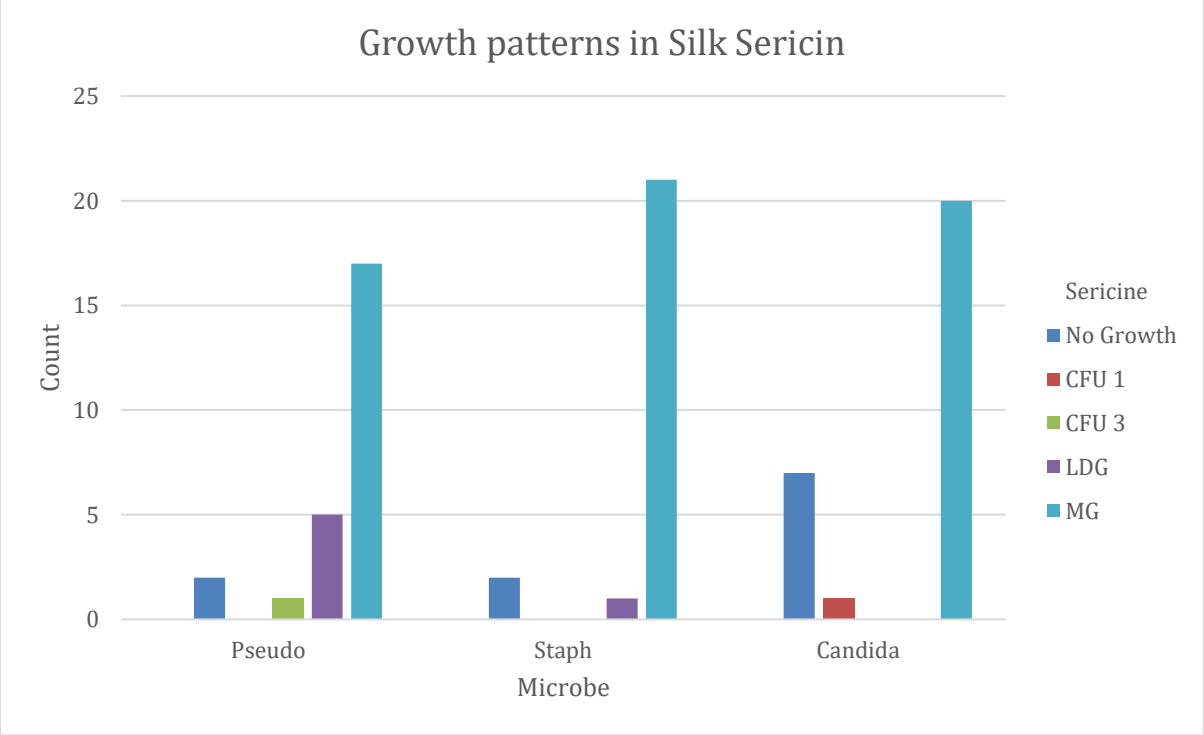

Figure 13:Growth patterns in Silk Sericin

CFU: Colony Forming Units, LDG: Less Dense Growth, MG: Moderate Growth

Blue bar – absolute count of microbes that showed no growth

Maroon bar – absolute count of microbes that showed CFU 1

Green bar – absolute count of CFU 3

Purple bar - absolute count of LDG

Sky blue bar - absolute count of moderate Growth

Growth proportion of *Pseudomonas* was 64% (16/25) in the 25 samples in Molasses while *Staphylococcus* had 62.5% (15/24) in the 24 samples. Additionally, 17.9% (5/28) of the 28 *Candida spp* samples had growth in Molasses. This is shown in figure 14 and table 5 below.

Table 5:Positive growth patterns in Molasses

Organism	Absolute count / Total samples	Percentage (%)
Pseudomonas	16/25	64
Staphylococcus	15/24	62.5
Candida	5/28	17.9

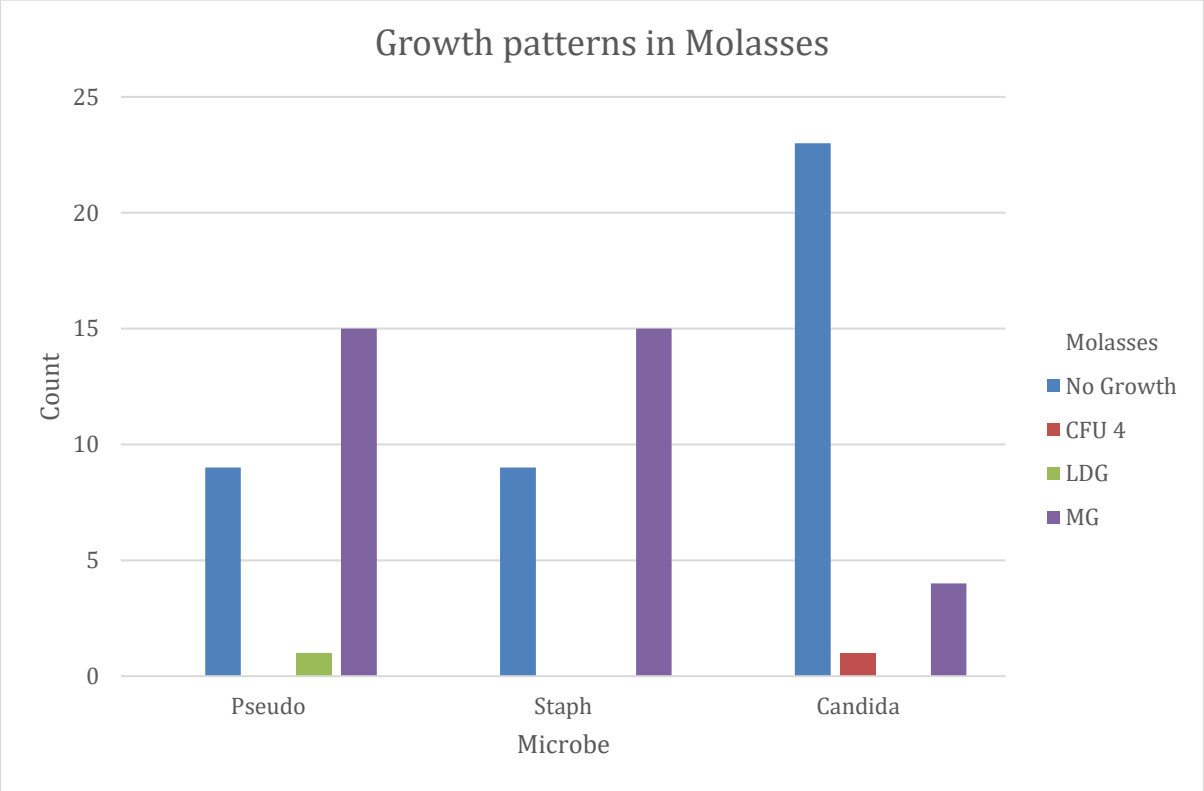

Figure 14:Growth patterns in Molasses

CFU: Colony Forming Units, LDG: Less Dense Growth, MG: Moderate Growth

Blue bar – absolute count of microbes that showed no growth

Maroon bar – absolute count of microbes that showed CFU 4

Green bar – absolute count of LDG

Purple bar - absolute count of MG

Growth was observed in all subcultures of *Pseudomonas* and *Staphylococcus* in the sugar broths. However, only 64.3% of Candida spp samples demonstrated presence of growth.

Table 6:Positive growth patterns in Sugar

Organism	Absolute count / Total samples	Percentage (%)
Pseudomonas	25/25	100
Staphylococcus	24/24	100
Candida	18/28	64.3

Growth rate and pattern of *pseudomonas aeruginosa* in Local honey was evenly distributed between moderate growth, less dense growth and four colony forming units (CFUs). 60% of growth in Molasses was moderate growth while 4% was less dense growth. In Sericin, 68% of growth was moderate while 20% and 4% were less dense growth and three CFUs respectively. These are represented by table 7 and figures 11-14.

Table 7:Distribution of growth characteristics of Pseudomonas in antimicrobials

Growth characteristic	Manuka Honey	Local Honey	Sugar	Molasses	Silk Sericin	Percentage (%)
1 CFU	0	0	0	0	0	
2 CFU	0	0	0	0	0	
3CFU	0	0	0	0	4	
4CFU	0	4	0	0	0	
LDG	0	4	0	4	20	
MG	0	4	100	60	68	
VDG	0	0	0	0	0	

CFU: Colony Forming Units, LDG: Less Dense Growth, MG: Moderate Growth, VDG: Very Dense Growth

Staphylococcal growth patterns and rate was 10.7% and 6% for moderate growth and three CFUs in local honey. Growth in molasses was 100% moderate but 75% in Sericin. Only 16.7% of the growth in Sericin was less dense growth. These are represented by table 8 and figures 11-14.

Table 8:Distribution of growth characteristics of Staphylococcus in antimicrobials

Growth characteristic	Manuka Honey	Local Honey	Sugar	Molasses	Silk Sericin	Percentage (%)
1 CFU	0	0	0	0	0	
2 CFU	0	0	0	0	0	
3CFU	0	6	0	0	0	
4CFU	0	0	0	0	0	
LDG	0	0	0	0	16.7	
MG	0	10.7	100	62.5	75	
VDG	0	0	0	0	0	

CFU: Colony Forming Units, LDG: Less Dense Growth, MG: Moderate Growth, VDG: Very Dense Growth

The growth pattern on *Candida spp* was exclusively moderate in Manuka honey. 10.7% of the samples had moderate growth while 7.2% had less dense growth and four CFUs, equally distributed in Local honey. 46.4% of growth in sugar broth was moderate while 17.9% had four CFUs. Of the samples with growth in molasses, 14.3% were moderate while 3.6% had four CFUs. One CFU was noted in 3.6% of the samples in Sericin while 71.4% had moderate growth. These are represented by table 9 and figures 11-14.

Table 9:Distribution of growth characteristics of Candida in antimicrobials

Growth characteristic	Manuka Honey	Local Honey	Sugar	Molasses	Silk Sericin	Percentage (%)
1 CFU	0	0	0	0	3.6	
2 CFU	0	0	0	0	0	
3CFU	0	0	0	0	0	
4CFU	0	3.6	0	0	0	
LDG	0	3.6	17.9	3.6	0	
MG	3.6	10.7	46.4	14.3	71.5	
VDG	0	0	0	0	0	

CFU: Colony Forming Units, LDG: Less Dense Growth, MG: Moderate Growth, VDG: Very Dense Growth

The figure 15 below summarizes the antimicrobial susceptibility of the different microorganisms in the different honey based compounds.

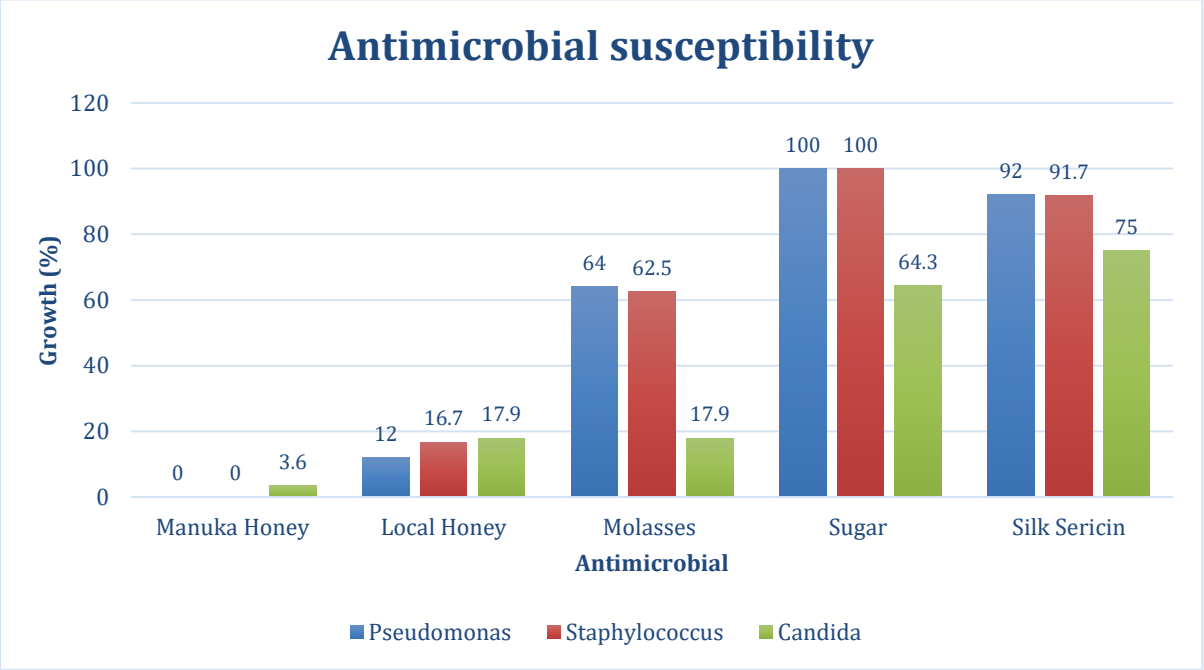

Figure 15:Summary of antimicrobial susceptibility

The general effect of the antimicrobials on the presence or absence of growth were analyzed. There was a significant difference on chi square analysis between the antimicrobial susceptibility of the three organisms to the antimicrobials under consideration; **p=0.000**, **p=0.000** and **p=0.000** for *pseudomonas*, s*taphylococcus* and *candida* respectively as shown in table 10 below.

Table 10:Antimicrobial susceptibility on chi square testing

Organism	Chi Square p-values
Pseudomonas	**0.000**
Staphylococcus	**0.000**
Candida	**0.000**

4.3 Minimum Inhibitory Concentration

With the tube dilution method, the MIC for *Pseudomonas aeruginosa* and *Staphylococcus aureus* was 25 %v/v in both Manuka honey and Local honey and 75 %v/v in both Molasses and Silk Sericin. *Candida spp* had an MIC of 50% v/v in Manuka honey, Local honey and Silk sericin and an MIC of 75% v/v in Silk Sericin. Growth was observed in all dilutions done with sugar within the limit of the study hence its MIC could not be established. There was a significant different in the MIC values of all the cultured organisms in all the antimicrobials (**p=0.000**). Table 11 and figure 16 summarize the results above.

Table 11:Median Minimum Inhibitory Concentrations (% v/v)

Organism	Manuka Honey	Local Honey	Molasses	Silk Sericin
Pseudomonas	25	25	75	75
Staphylococcus	25	25	75	75
Candida	50	50	50	75

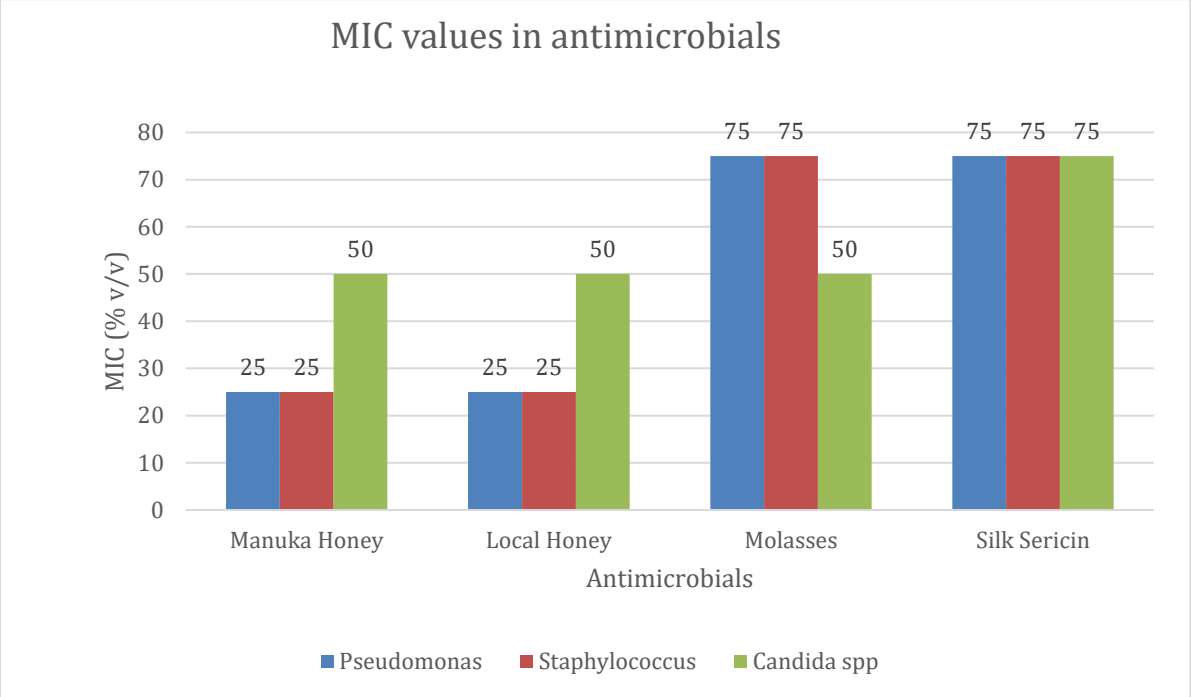

Figure 16:Median Minimum inhibitory concentrations

4.4 Minimum Bactericidal Concentration

The MBC of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was 50% v/v in both Manuka honey and Local honey and 100% v/v in both Molasses and Silk Sericin. Candida spp had an MBC of 75% v/v in Manuka honey, Local honey and Molasses and an MBC of 100% v/v in Silk Sericin. There was a significant different in the MBC values of all the cultured organisms in all the antimicrobials (**p=0.000**). These results are summarized in table 12 and figure 17 below.

Table 12:Median Minimum Bactericidal Concentrations (% v/v)

Organism	Manuka Honey	Local Honey	Molasses	Silk Sericin
Pseudomonas	50	50	100	100
Staphylococcus	50	50	100	100
Candida	75	75	75	100

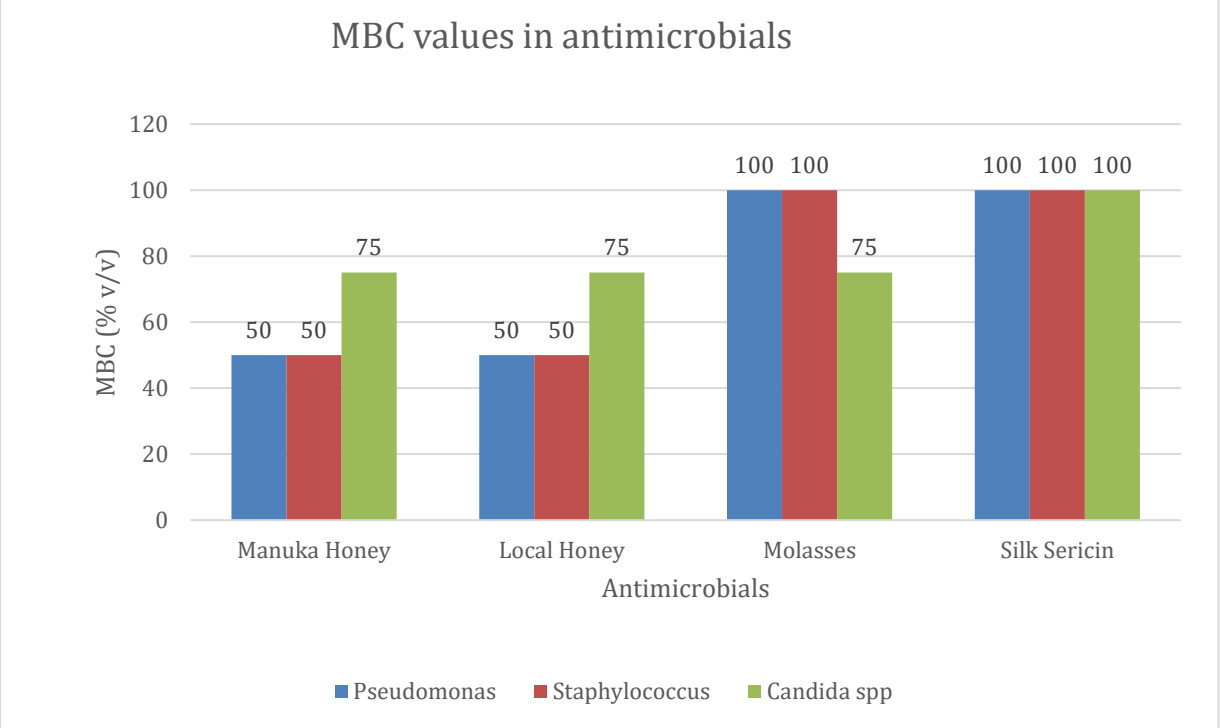

Figure 17: Median Minimum bactericidal concentrations

5.0 CHAPTER FIVE: DISCUSSION, CONCLUSION & RECOMMENDATIONS

5.1 Discussion

5.1.1 Antimicrobial Susceptibility

Bacterial colonization of wounds remains a major challenge in the management of wounds from whatever the cause. The rise in the antibiotic resistant rates of different microorganisms has compounded the difficulties encountered in management of wounds using the conventional antibiotics, besides the financial burdens (Albaridi, 2019). It is with this fact that natural products that is, plant and plant-based products with antimicrobial properties such as honey and honey-based products have been advocated for, because of their availability and affordability. Besides, no resistance to natural products have been reported thus far (Basualdo et al., 2007; Mandal and Mandal, 2011). The antimicrobial properties of honey have been known for centuries and have been used for therapeutic purposes in many cultures. Honey contains various natural substances such as hydrogen peroxide, flavonoids, and phenolic acids that can inhibit the growth of microorganisms. Similar mode of action is postulated for honey-based compounds such as Molasses, Sugar and Silk Sericine (Albaridi, 2019). In our study, results showed variable growth patterns observed with Silk Sericine and Molasses with increased susceptibility seen with Molasses compared to Silk Sericine. Manuka honey was superior to local honey in its antimicrobial properties. Overall honey was superior to sugar , molasses and sericin.

5.1.2 Minimum Inhibitory and Bactericidal Concentrations

Manuka and Local honey showed potent antimicrobial effects (MIC/MBC: Pseudomonas/Staphylococcus 25%/50%, Candida spp 50%/75%). Molasses and Silk Sericin had moderate effects (MIC/MBC: Pseudomonas/Staphylococcus 75%/100%). Candida spp was more susceptible to Molasses (MIC/MBC: 50%/75%) than Silk Sericin (MIC/MBC: 75%/100%). Sugar had the weakest antimicrobial effect. These findings indicate that Manuka honey and Local honey are effective antimicrobials against *pseudomonas*, *staphylococcus aureus*, and *candida*. These results are consistent with previous studies that have shown the antimicrobial properties of honey(Mandal and Mandal, 2011). The potency of Manuka honey is due to its high content of methylglyoxal, which is a potent antimicrobial compound. This activity, together with its Methyl Syringate activity makes it a potent antimicrobial even after its hydrogen peroxide actions are inhibited hence also referred to as a "non-peroxide honey" (Basualdo et al., 2007).Local honey, on the other hand, contains a variety of natural substances that have antimicrobial properties, including hydrogen peroxide, flavonoids, and phenolic

acids. These compounds have been showed to act as potent antioxidants and may have antimicrobial properties (Albaridi, 2019).

Additionally, honey contains natural sugars such as fructose and glucose, which are thought to create an osmotic effect that draws water out of bacterial cells, leading to their dehydration and ultimately their death (Cooper et al., 2002a). Honey also contains trace amounts of minerals such as calcium, magnesium, and potassium, which may also contribute to its antimicrobial properties.

The low pH of honey is another factor that contributes to its antimicrobial activity. The pH of honey is typically between 3.2 and 4.5, which is too acidic for many microorganisms to survive. Moreover, honey promotes the release of inflammatory cytokines from monocytes which is pivotal in healing and repair of wounds (Tonks et al., 2001, 2003). These factors come together to make honey an excellent alternative to conventional therapy in the treatment of burn wounds, infected surgical wounds and even gastric and peptic ulcers (Adinortey et al., 2022; Albaridi, 2019; Mandal et al., 2010). Studies have shown that honey is effective against *Pseudomonas*, *E coli*, MRSA, Vancomycin resistant Enterococci (VRE) as well as gram positive cocci (Adinortey et al., 2022; Ahmed et al., 2003; Cooper et al., 2002a, 2002b)

The levels of inhibition and killing kinetics of these honey-based products differ with geographical location and source of the individual constituents. French et al., reported a MIC of 3.4 with coagulase negative *Staph* , Basson and Grobler a MIC of 25 with *Staph* and *E coli* while Cooper et al., reported MIC of 3 with *Staph* (Basson and Grobler, 2008; Cooper et al., 1999; French et al., 2005).

Molasses and Silk Sericin were found to have moderate antimicrobial effects, which may be due to their high sugar content. Sugars can inhibit microbial growth by drawing water out of microorganisms, thereby dehydrating them. However, the sugar concentration in Molasses and Silk Sericin may not be high enough to have a strong antimicrobial effect (El-Fakharany et al., 2020; Grabek-Lejko and Tomczyk-Ulanowska, 2013).

The weak antimicrobial effect of sugar is consistent with previous studies that have shown that sugar is not an effective antimicrobial. This is because most microorganisms can utilize sugars as a source of energy, so the presence of sugar may even promote microbial growth (Selwyn and Durodie, 1985).

Therefore, Manuka honey was superior to the other antimicrobials tested. This is as a result of its higher MIC and MBC. Local honey could also be useful as it is far superior to sugar, molasses and sericin.

5.2 Conclusion

Manuka honey and Local honey are effective antimicrobials against *pseudomonas*, *staphylococcus aureus*, and *candida*. Molasses and Silk Sericin have moderate antimicrobial effects, while sugar has a weak antimicrobial effect. These findings suggest that honey-based products may have best potential as natural antimicrobial agents. They offer an alternative effective agent for wound management not only in chronic wounds but also in cases of wounds infected with drug resistant strains of organisms. In terms of availability and cost, local honey is easily accessible and affordable compared to Manuka honey. We therefore recommend that local honey should be routinely used in the management of chronic wound as well as infected wounds refractory to conventional therapy.

MIC and MBC provide valuable information about a drug's effectiveness against microorganisms, but they are not the sole determinants of dosage. Dosage determination involves a comprehensive evaluation of factors such as pharmacokinetics, pharmacodynamics, therapeutic objectives, and patient-specific considerations.

Nonetheless, based purely on our MIC and MBC results, we suggest minimum dilutions of 75% v/v of both local honey and Manuka honey in volume in wounds infected primarily by Candida species. As for wounds infected by pseudomonas aeruginosa and staphylococcus aureus, we recommend dilutions of 50% v/v of both local honey and Manuka honey.

5.3 Recommendations

While the current study has shed light on the antimicrobial effects Manuka honey, Local honey, Molasses and Silk Sericin, there are still several gaps in the literature that need to be addressed. Although the antimicrobial effects of Manuka honey and local honey have been demonstrated in vitro, further clinical trials are needed to determine their efficacy in humans. This would include investigating the optimal dosages and treatment regimens for different types of infections caused by Pseudomonas aeruginosa, Staphylococcus aureus and Candida.

The exact mechanism by which Manuka honey and local honey exert their antimicrobial effects is not fully understood. Further research is needed to elucidate the specific compounds responsible for these effects, and the pathways by which they act on the pathogens.

Manuka honey and local honey have been shown to have potent antimicrobial effects, but it is unclear how they compare with conventional antibiotics in terms of efficacy, safety and tolerability. Further research such as randomized clinical trial is needed to compare the efficacy of honey with conventional antibiotics in the treatment of infections caused by Pseudomonas aeruginosa, Staphylococcus aureus and Candida.

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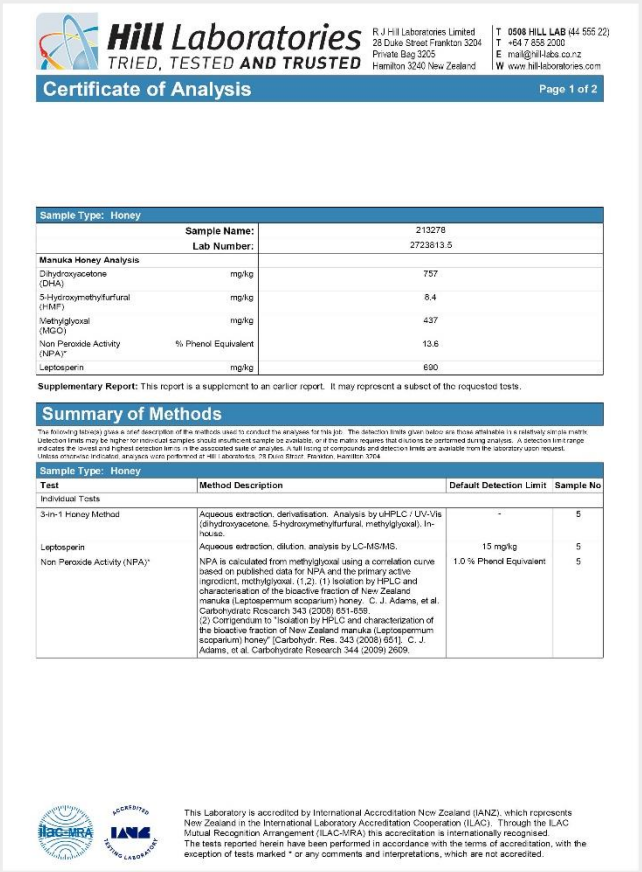
APPENDICES

Appendix I: Manuka Honey Certificate of Analysis;

BATCH NO 213278



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R J Hill Laboratories Limited
28 Duke Street Frankton 3204
Private Bag 3205
Hamilton 3240 New Zealand

T 0508 HILL LAB (44 555 22)
T +64 7 858 2000
E mail@hill-labs.co.nz
W www.hill-laboratories.com

Certificate of Analysis Page 1 of 2

Sample Type: Honey		
	Sample Name:	213278
	Lab Number:	2723813.5
Manuka Honey Analysis		
Dihydroxyacetone (DHA)	mg/kg	757
5-Hydroxymethylfurfural (HMF)	mg/kg	8.4
Methylglyoxal (MGO)	mg/kg	437
Non Peroxide Activity (NPA)*	% Phenol Equivalent	13.6
Leptosperin	mg/kg	690

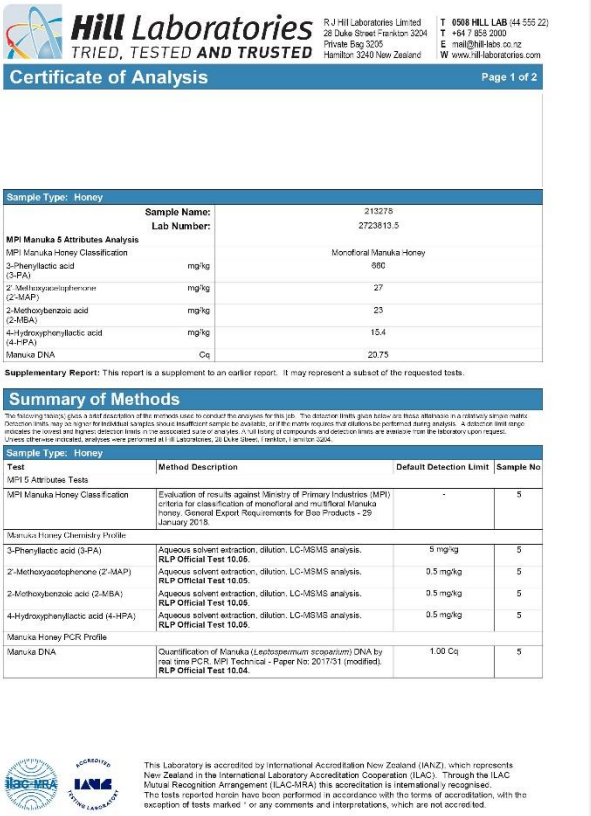
Supplementary Report: This report is a supplement to an earlier report. It may represent a subset of the requested tests.

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively simple matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. A detection limit range indicates the lowest and highest detection limits in the associated suite of analytes. A full listing of compounds and detection limits are available from the laboratory upon request. Unless otherwise indicated, analyses were performed at Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Honey			
Test	**Method Description**	**Default Detection Limit**	**Sample No**
Individual Tests			
3-in-1 Honey Method	Aqueous extraction, derivatisation. Analysis by uHPLC / UV-Vis (dihydroxyacetone, 5-hydroxymethylfurfural, methylglyoxal). In-house.	-	5
Leptosperin	Aqueous extraction, dilution, analysis by LC-MS/MS.	15 mg/kg	5
Non Peroxide Activity (NPA)*	NPA is calculated from methylglyoxal using a correlation curve based on published data for NPA and the primary active ingredient, methylglyoxal. (1,2). (1) Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (Leptospermum scoparium) honey. C. J. Adams, et al. Carbohydrate Research 343 (2008) 651-659. (2) Corrigendum to "Isolation by HPLC and characterization of the bioactive fraction of New Zealand manuka (Leptospermum scoparium) honey" [Carbohydr. Res. 343 (2008) 651]. C. J. Adams, et al. Carbohydrate Research 344 (2009) 2609.	1.0 % Phenol Equivalent	5

This Laboratory is accredited by International Accreditation New Zealand (IANZ), which represents New Zealand in the International Laboratory Accreditation Cooperation (ILAC). Through the ILAC Mutual Recognition Arrangement (ILAC-MRA) this accreditation is internationally recognised. The tests reported herein have been performed in accordance with the terms of accreditation, with the exception of tests marked * or any comments and interpretations, which are not accredited.



Hill Laboratories
TRIED, TESTED AND TRUSTED

R J Hill Laboratories Limited
28 Duke Street Frankton 3204
Private Bag 3205
Hamilton 3240 New Zealand

T 0508 HILL LAB (44 555 22)
T +64 7 858 2000
E mail@hill-labs.co.nz
W www.hill-laboratories.com

Certificate of Analysis Page 1 of 2

Sample Type: Honey		
	Sample Name:	213278
	Lab Number:	2723813.5
MPI Manuka 5 Attributes Analysis		
MPI Manuka Honey Classification		Monofloral Manuka Honey
3-Phenyllactic acid (3-PA)	mg/kg	660
2'-Methoxyacetophenone (2-MAP)	mg/kg	27
2-Methoxybenzoic acid (2-MBA)	mg/kg	23
4-Hydroxyphenyllactic acid (4-HPA)	mg/kg	15.4
Manuka DNA	Cq	20.75

Supplementary Report: This report is a supplement to an earlier report. It may represent a subset of the requested tests.

Summary of Methods

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively simple matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. A detection limit range indicates the lowest and highest detection limits in the associated suite of analytes. A full listing of compounds and detection limits are available from the laboratory upon request. Unless otherwise indicated, analyses were performed at Hill Laboratories, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Honey			
Test	**Method Description**	**Default Detection Limit**	**Sample No**
MPI 5 Attributes Tests			
MPI Manuka Honey Classification	Evaluation of results against Ministry of Primary Industries (MPI) criteria for classification of monofloral and multifloral Manuka honey. General Export Requirements for Bee Products - 29 January 2018.	-	5
Manuka Honey Chemistry Profile			
3-Phenyllactic acid (3-PA)	Aqueous solvent extraction, dilution, LC-MSMS analysis. **RLP Official Test 10.05.**	5 mg/kg	5
2'-Methoxyacetophenone (2-MAP)	Aqueous solvent extraction, dilution, LC-MSMS analysis. **RLP Official Test 10.05.**	0.5 mg/kg	5
2-Methoxybenzoic acid (2-MBA)	Aqueous solvent extraction, dilution, LC-MSMS analysis. **RLP Official Test 10.05.**	0.5 mg/kg	5
4-Hydroxyphenyllactic acid (4-HPA)	Aqueous solvent extraction, dilution, LC-MSMS analysis. **RLP Official Test 10.05.**	0.5 mg/kg	5
Manuka Honey PCR Profile			
Manuka DNA	Quantification of Manuka (*Leptospermum scoparium*) DNA by real-time PCR, MPI Technical - Paper No: 2017/31 (modified). **RLP Official Test 10.04.**	1.00 Cq	5

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Appendix II: Local honey (Meru) Certificate of analysis

REPUBLIC OF KENYA

MINISTRY OF AGRICULTURE, LIVESTOCK AND FISHERIES

NATIONAL BEEKEEPING INSTITUTE

Certificate of Honey Analysis

This is to certify that a Laboratory Analysis was carried out on the following Honey sample using harmonized methods of the international honey commission and the results were as follows

A. PARTICULARS:

Sample Code No	040/23
Date Collected	15/3/2023
Origin	MERU
Name of producer/packer	KARUMO TTI
Address	N/A
Tel	+254721602278

B. PHYSICAL ANALYSIS

Colour	AMBER
Condition	LIQUID
Packaging material	PLASTIC
Label	SAMPLE A
B/N	N/A

C. CHEMICAL ANALYSIS

NO	COMPONENTS	RECOMMENDED % CONTENT	RESULTS	OUTCOME PASS/FAILED
1.	WATER MOISTURE	NOT MORE THAN 20%	17.2	PASS
2.	TOTAL REDUCING SUGAR(TRS)	NOT LESS THAN 65%	77.0	PASS
3.	APPARENT SUCROSE	NOT MORE THAN 5%	0.3	PASS
4.	ACIDITY	NOT MORE THAN 40 mg/kg	36	PASS
5.	HYDROXYMETHYLFURFURAL (HMF)	NOT MORE THAN 40mg/kg	1.5	PASS

REMARKS THE HONEY SAMPLE HAS COMPLIED WITH THE SET HARMONISED INTERNATIONAL STANDARDS

Certified at the NATIONAL BEEKEEPING INSTITUTE, P. O. Box 34188(00100) , Tel: 3864302/1, Nairobi, KENYA.

Analyst.....

Date Analyzed: 5/4/23

Principal

Head: Quality Control Section

This certificate was issued without any alterations.

This certificate is valid for this consignment only

Appendix III: Ethical Approval Letter

UNIVERSITY OF NAIROBI
FACULTY OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/46

30th January, 2023

KENYATTA NATIONAL HOSPITAL APPROVED 30 JAN 2023 KNH/UoN-ERC P.O. Box 20723 - 00202 NAIROBI

Dr. Robert Kyalo Mbaluka
Reg. No. H58/1136/2018
Dept. of Surgery
Faculty of Health Sciences
University of Nairobi

Dear Dr. Mbaluka,

RESEARCH PROPOSAL: COMPARISON OF THE ANTIMICROBIAL EFFECTS OF MANUKA HONEY, LOCAL HONEY, SUGAR, MOLASSES AND SILKWORM SERICINS ON COMMONLY ISOLATED WOUND MICROBES (P732/09/2022)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P732/09/2022.** The approval period is 30th January 2023 – 29th January 2024.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Protect to discover

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

DR. BEATRICE K.M. AMUGUNE
SECRETARY, KNH-UoN ERC

c.c. The Dean, Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Assistant Director, Health Information Dept., KNH
The Chairperson, KNH- UoN ERC
The Chair, Dept. of Surgery, UoN
Supervisors: Dr. Ferdinand Nang'ole, Dept. of Surgery, UoN
Dr. Marianne W. Mureithi, Dept. of Medical Microbiology, UoN
Dr. Adan Abdullahi, Dept. of Surgery, UoN
Dr. Angela Muoki, Dept. of Surgery, UoN

Appendix IV: NACOSTI Research License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 525125

Date of Issue: 11/May/2023

RESEARCH LICENSE

This is to Certify that Dr.. Robert Mbaluka of University of Nairobi, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nairobi on the topic: COMPARISON OF THE ANTIMICROBIAL EFFECTS OF MANUKA HONEY, LOCAL HONEY, SUGAR, MOLASSES AND SILKWORM SERICINS ON COMMONLY ISOLATED WOUND MICROBES for the period ending : 11/May/2024.

License No: NACOSTI/P/23/25786

525125

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

27/11/23.
Dr. M. Muralli

Comparison of The Antimicrobial Effects Of Manuka Honey, Local Honey, Sugar, Molasses And Silkworm Sericins On Commonly Isolated Wound Microbes

27 NOV. 2023

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