COMPARATIVE EFFICACY OF STINGLESS BEE (KELULUT) HONEY AND MEDICAL-GRADE MANUKA HONEY FOLLOWING MAGGOT DEBRIDEMENT THERAPY IN DIABETIC FOOT ULCER TREATMENT

BY

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ABSTRACT

Diabetes-based foot complications have become a healthcare burden to many developing countries where 25% of diabetics with poorly controlled diabetes tend to develop diabetic foot ulcer (DFU) in their lifetime. DFU is resistant to conventional methods and takes a longer time to heal. Medical-grade manuka honey has been proven to show positive wound healing outcomes and is used alternatively in healthcare settings. However, research on stingless bee (kelulut) honey has not been forthcoming. Thus, this study was conducted to evaluate the efficacy of kelulut honey in the healing of DFU as compared to medical-grade manuka honey based on the ulcer size reduction from baseline to days 7 and 14. Maggot debridement therapy (MDT) using sterile maggots of local species *Lucilia cuprina* was utilised as the debridement modality in this study. Quasi-experimental study design was applied to perform the study in University Malaya Medical Centre (UMMC) involving 60 participants with DFU who were divided into two groups receiving kelulut honey (Group 1) and medical-grade manuka honey (Group 2). Wound measurements were done at baseline, day 7, and day 14 using a mobile wound monitoring application system, NDKare^{TM}. Statistical analysis with Mann-Whitney U test yielded a statistically significant percentage of ulcer size reduction ($p<0.001$) on day 14 between the kelulut honey group and medical-grade manuka honey group (47.10% vs 7.5%). The findings indicated that kelulut honey is more effective than medical-grade manuka honey and advocated kelulut honey as a potential wound dressing to improve the healing of DFU.

Keywords: honey, diabetic foot ulcer, stingless bee, manuka

البحث ملخص

أصبحت مضاعفات القدم القائمة على مرض السكري عبئًا على الرعاية الصحية للعديد من البلدان النامية حيث يميل 25% من مرضى السكري الذين يعانون من ضعف السيطرة على مقاوم DFU .في حياتهم (DFU) مرض السكري إلى الإصابة بقرحة القدم السكرية للطرق التقليدية ويستغرق وقتًا أطول للشفاء. لقد ثبت أن عسل مانوكا الطبي يظهر نتائج إيجابية لشفاء الجروح ويستخدم بدلاً من ذلك في أماكن الرعاية الصحية. ومع ذلك، لم يتم إجراء أبحاث حول عسل النحل غير اللاسع (كيلولوت). وهكذا، أجريت هذه الدراسة لتقييم مقارنة بعسل المانوكا الطبي بناءً على تقليل حجم DFU فعالية عسل الكيلولوت في شفاء (MDT) القرحة من خط الأساس إلى اليومين 7 و14. تم استخدام علاج تنضير اليرقات كطريقة للتنضير في *Lucilia cuprina* باستخدام الديدان المعقمة من الأنواع المحلية هذه الدراسة. تم تطبيق تصميم دراسة شبه تجريبية لإجراء الدراسة في المركز الطبي الذين تم تقسيمهم إلى DFU والتي شملت 60 مشاركًا من (UMMC) بجامعة مالايا مجموعتين يتلقون عسل الكيلولوت (المجموعة 1) وعسل المانوكا الطبي (المجموعة 2). تم إجراء قياسات الجروح عند خط الأساس، اليوم 7، واليوم 14 باستخدام نظام تطبيق أسفر التحليل الإحصائي باستخدام اختبار .NDKare™ ،مراقبة الجروح المحمول عن نسبة ذات دلالة إحصائية من انخفاض حجم القرحة Mann-Whitney U في اليوم 14 بين مجموعة عسل كيلولوت ومجموعة عسل مانوكا الطبية ($p<0.001$) (47.10% مقابل 7.5%). أشارت النتائج إلى أن عسل الكيلولوت أكثر فعالية من عسل المانوكا الطبي، ودعت إلى عسل الكيلولوت كضمادة محتملة للجروح لتحسين شفاء DFU.

الكلمات المفتاحية: العسل، قرحة القدم السكرية، النحل غير اللاسع، مانوكا

APPROVAL PAGE

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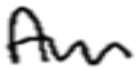

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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List of Abbreviations

ABPI	Ankle Brachial Pressure Index
ADA	American Diabetes Association
BMI	Body Mass Index
CKD	Chronic Kidney Disease
DFU	Diabetic Foot Ulcer
DHA	Dihydroxyacetone
HUKM	Hospital University Kebangsaan Malaysia
Hb	Hemoglobin
HbA1c	Glycated hemoglobin A1c
IDSA	Infectious Disease Society of America
IDF	International Diabetes Federation
IIUM	International Islamic University of Malaysia
IWGDF	International Working Group Diabetic Foot
KON	Kulliyyah of Nursing
MDT	Maggot Debridement Therapy
MGO	Methylglyoxal
MREC	Medical Research Ethics Committee
NGSP	National Glycohemoglobin Standardisation Program
NHMS	National Health and Morbidity Survey
NMRR	National Medical Research Registrar
ROS	Reactive Oxygen Species
T.I.M.E	Tissue, Inflammation, Moisture balance, Epithelial Advancement Framework
USFDA	United States Food and Drug Administration
UMMC	University Malaya Medical Centre
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION TO THE STUDY

1.1 INTRODUCTION

Prior to the discovery of antibiotics, natural remedies using insects and their products have been used as healing agents in many parts of the globe since ancient times (Izzah Ibrahim et al., 2018). Flies and bees were prominently used in the prevention of illness and management of diseases centuries ago (Carina Biluca et al., 2021). However, the introduction and administration of antibiotics, such as penicillin, have reduced the use of ancient remedies in the treatment of diseases. Consequently, the spread of broad-spectrum antibiotics brought forward the alarming emergence of methicillin-resistant *Staphylococcus aureus* in the late 1980s (Kwon & Armstrong, 2018). Antibiotic resistance created a huge challenge for clinicians to manage infections of chronic illness and wounds especially diabetic foot ulcers (DFU) (Zubair, 2020). The delay in the healing of DFU can lead to serious, devastating foot complications, which have largely contributed to prolonged hospitalisations and lower limb amputations and greatly affected patients' quality of life (Ng et al., 2017). Given the impact of DFU on the clinical and social aspects, honey made a comeback into modern wound care to enhance healing outcomes (Lavinas et al., 2019; Mama, Teshome, & Detamo, 2019; Mavrogenis et al., 2018; Saikaly & Khachemoune, 2017). However, there have been limited studies highlighting the significance of honey in the treatment of DFU. With much prominence given to manuka honey-based wound dressings, honey produced by stingless bees has not been much explored. The present study was undertaken to explore the efficacy of local stingless bee kelulut honey in the treatment of DFU as compared to medical-grade manuka honey. This chapter mainly describes the background of the study and the problem statement, followed by an elaboration of the research objectives, research questions, and the significance of the study.

1.2 BACKGROUND OF THE STUDY

Diabetes mellitus is a chronic disease characterised by elevated levels of glucose in the blood known as hyperglycemia. The most common type of diabetes among the world's adult population is type 2 diabetes, which occurs when the body produces insufficient insulin, ineffective insulin, or both (Monteiro-Soares et al., 2020). According to Lin et al. (2020), diabetes is one of the largest global public health concerns, imposing a heavy global burden on public health as well as socio-economic development. Although incidence has started to decrease in some countries, the prevalence of diabetes has increased across other developed and developing countries in recent decades. Based on a recent report by the International Diabetes Federation (IDF), the prevalence of diabetes has escalated dramatically in the past three decades in countries of all income levels with more than 10.5% of the adult population across the globe suffering from diabetes. Furthermore, diabetes has been highlighted as one of the major non-communicable diseases (NCDs) worldwide that has affected 463 million adults (20-79 years). This number is expected to rise to 783 million (12.2%) in 2045 (IDF, 2021). According to World Health Organization (WHO), the prevalence of diabetes is highly alarming whereby 1 in 5 people in the adult population have diabetes and about 1.6 million deaths have been documented annually among the adult diabetic population. A sedentary lifestyle and an aging population have largely contributed to the rise of diabetes worldwide. Likewise, the National Health and Morbidity Survey (NHMS) conducted in a 4-year cycle in Malaysia reported that 3.9 million adults over 18 years are suffering from diabetes, which accounts for 18.3% in a population of 32.6 million. The report signifies that the prevalence of diabetes in Malaysia is considered significantly higher than its neighboring countries such as Singapore and Indonesia, with 1 out of 5 people in Malaysia being diabetic (NHMS, 2019). Based on the NHMS (2019) report, Figure 1 illustrates the increasing trend in the prevalence of diabetes from 11.6% in 2006 to 18.3% in 2019 in Malaysia.

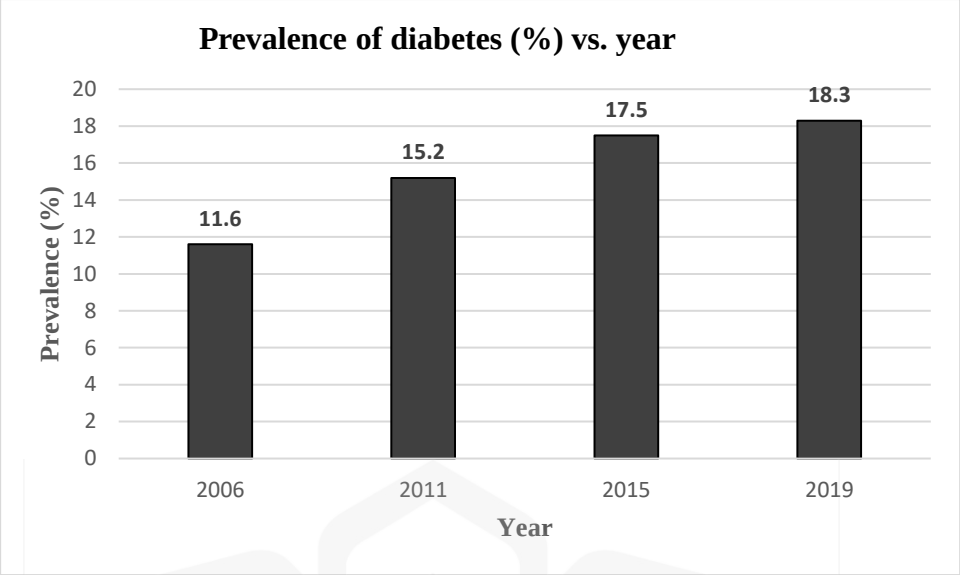

Figure 1: Prevalence of Diabetes in Malaysia (2006-2019)

The burden of diabetes and its complications has greatly affected the healthcare system worldwide and the budget has increased tremendously over the years. The healthcare burden of diabetes cannot be underestimated in Malaysia either. As mentioned in the NHMS (2019) report, the estimated cost of managing diabetes was RM2.04 billion with the government sector absorbing RM1.4 billion of the cost, representing 9.21% of the Ministry of Health's total budget. Due to the increasing trend of diabetes, the healthcare budget was estimated to rise as well, which could expand the financial burden for a developing country such as Malaysia. As the world witnessed a sharp rise in the prevalence of diabetes among the adult population, the incidences of DFU complications were increasing as well due to poor glycemic control (Boulton et al., 2020; Chun et al., 2019).

The definition of DFU was established decades ago but has been redefined in recent times as an open, full-thickness wound located below the ankle with either acute or chronic etiology. A chronic DFU is described as a wound of more than 2 weeks old whereas acute DFU is less than 2 weeks. When a DFU does not heal with conventional or standard methods in 3 months, it is termed a non-healing chronic DFU (Coffey et al., 2019; Thewjitcharoen et al., 2014). DFU is a debilitating consequence of diabetes with a lifetime incidence of up to 25% and has been described as one of the most common and worst

complications of poorly controlled diabetes across the globe. According to (Gandhi et al. (2019), vascular insufficiency and peripheral neuropathy also play a major role in the occurrence of foot ulcers among diabetics. The complication of DFU includes infection, gangrene, and lower limb or digit amputations. Recent reports indicated that 80% of non-traumatic lower-limb amputations are preceded by DFU (Everett & Mathioudakis, 2018). Non-healing chronic DFU has also become a common cause of prolonged hospital admissions worldwide whereby 10% of all hospital admissions were contributed by infected DFUs (Boulton, 2019; Hopkins et al., 2015; Pemayun & Naibaho, 2017; Skrepnek et al., 2017). Worst still, previous reports revealed that the infection rate for DFU was 41% and death preceding complicated DFU was 50% within the past 5 years, with 70% preceding amputations (Adiewere et al., 2018; Mavrogenis et al., 2018; Ndosi et al., 2018; Raghav et al., 2018).

DFU also greatly affects the patient's quality of life and loss of productivity especially due to lower limb amputations, which account for 6-8% among the 25% of diabetics who developed DFU. This number is expected to rise with the escalating prevalence of diabetics in the country due to unhealthy lifestyles (Letchuman et al., 2010; Makhtar, 2016; Tee & Yap, 2017). The prevalence of DFU in Malaysia ranged high from 5–10%, and only in Kuala Lumpur, the prevalence of DFU reached as high as 42%. Statistics related to the prevalence of DFU and the associated consequences were alarming (Kee et al., 2019; Nair et al., 2022). According to the International Working Group for Diabetic Foot (IWGDF), more than half of DFUs develop an infection and 17% would require amputation. Nevertheless, for patients whose wounds were healed, recurrence was predominantly present in 40% of them within a year, 65% within 5 years, and more than 90% within 10 years. Apart from that, the treatment cost related to DFU management was escalating as well (IWGDF, 2019). The study by Ousey et al. (2018) highlighted that the annual management cost of infected DFU is high and was estimated at USD 11,000, which included the administration of antibiotics (50%), surgical procedure (9%), wound dressing (18%), as well as admission and baseline investigation (23%). Undoubtedly, the treatment for foot complications has long been known as a financial burden to developing countries (Bakker et al., 2016).

Hence, the management of DFUs becomes a baffling challenge to clinicians worldwide as current treatments are unable to produce targeted outcomes to cripple the complication of DFUs. Furthermore, loss of limb is reported every 20 seconds across the globe due to diabetes-based foot complications, which sets the alarm on the irreversible impact of diabetes-based foot global disability if the matter is not treated efficiently and promptly (Everett & Mathioudakis, 2018; Guest et al., 2018). The systematic approach to managing and treating DFU includes infection control, restoration of blood flow, efficient debridement, appropriate dressing, exudate control, continuous infection control, and offloading (Boulton et al., 2018). As DFU is gaining resistance towards conventional methods and its closure only accounts for 25-50% in 6-7 months (Masiero & Thyssen, 2016) , the search for alternative treatment is highly warranted to prevent serious complications, compromised quality of life, and loss of limbs (Doğruel et al., 2022; Ugwu et al., 2019). The benefits of using alternative treatment in the management of DFUs have gained momentum in the last two decades (Samarghandian et al., 2017). One such treatment is maggot debridement therapy (MDT), which has been successful to yield superior results compared to non-surgical conventional results in debriding sloughy diabetic foot ulcers and improve healing rates. MDT is the application of sterile medical-grade maggots on sloughy and necrotic wounds as an alternative debridement method in chronic wound healing (Marimuthu & Makhtar, 2020; El-Tawdy et al., 2016; Jordan et al., 2018; Mirabzadeh et al., 2017; Sherman, 2014; Tian et al., 2013). Honey is another nature-based biological remedy that has been valued for its therapeutic properties in wound healing.

Honey is a sweet natural substance produced by bees and is widely consumed for its nutritional value in boosting immunity against illnesses (Al-Hatamleh et al., 2020; Chuttong et al., 2016; Henshaw et al., 2014; Sousa et al., 2016). It has been consumed for decades to boost immunity against infection and overall well-being as scientific evidence shows that honey has more than 200 components of amino acids, enzymes, vitamins, carbohydrates, and minerals depending on the origin of the nectar. However, the composition of honey is highly dependent on the plant (origin of nectar) and geographical sites, such as manuka honey, acacia, buckwheat, or clover (Elbanna et al., 2014) Hence, different types of honey vary in their compositions, therapeutic efficacies, and safety (Rossi & Marrazzo, 2021). As the interest and knowledge in the therapeutic properties of honey

increases, the 21st century witnesses the global expanding usage of honey from a superfood to a potential alternative wound dressing in managing chronic conditions. Several studies have shown that honey has anti-bacterial, anti-inflammatory, and antioxidant properties which can be useful to overcome the surge of antibiotic resistance in chronic wounds, especially DFU (Kus & Ruiz, 2020; Meo et al., 2017; Münstedt, 2022). According to Cooper (2014), the beneficial therapeutic potentials of honey may play a major role in stimulating and accelerating the healing of chronic wounds. Thus, the therapeutic properties of honey have brought it to the forefront of wound management as a potential wound dressing (Biglari et al., 2013; Kateel et al., 2016; Lima et al., 2020; Mekkaoui et al., 2022). Consequently, medical-grade manuka honey has been incorporated as an alternative wound dressing for the treatment of burns and chronic wounds in recent times (Jull et al., 2015; Saikaly & Khachemoune, 2017; Yaghoobi et al., 2013).

Nevertheless, the majority of medical-grade honey used for medical purposes originated from manuka honey, which is produced by honey bees *Apis mellifera*. Manuka honey is one type of unifloral honey that has been widely researched and recognised internationally as a potential wound dressing for the treatment of burns and wounds (Frydman et al., 2020; Grajales-Conesa et al., 2017). Several studies have compared the antibacterial properties of manuka honey and other native honey in different geographical locations. However, the results were inconsistent with some studies showing a higher effect of antibacterial properties in manuka honey while others reporting otherwise or comparable outcomes (Aina et al., 2018; Aryani et al., 2020). Given the majority of research conducted on manuka honey, the United States of America Food and Drug Association (USFDA) approved the registration of manuka honey for the healing of burns and wounds in 2015. To date, manuka honey has been used as wound dressing either in its original form or processed or impregnated in gauze dressing for wound healing. However, only a handful of investigations demonstrated significant wound-healing outcomes in the treatment of DFU (Tashkandi, 2021; Xu Tian et al., 2014; Wang et al., 2019).

Despite the availability of honey from other alternative sources, such as stingless bee *Trigona sp* and *Mellipona sp*, acacia, buckwheat, and Tualang honey which are abundant in tropical countries, manuka honey is prominently considered an alternative

wound dressing (Ranneh et al., 2018; Zulkhairi Amin et al., 2018a). However, there are upcoming findings on the potential of non-manuka honey with similar therapeutic properties that could be potentially beneficial in the healing of DFU (Hewett et al., 2022). One type of bee which gained the interest of beekeepers in recent times to produce a substantial amount of honey is the stingless bee *Trigona sp* and *Mellipona sp* (Ismail & Ismail, 2018). Some studies have demonstrated the superior therapeutic anti-oxidant capacity of stingless bee honey which could be utilised for wound healing in the treatment of DFU (Abd Jalil et al., 2017; Al-Waili et al., 2011; Alvarez-Suarez et al., 2018; Esa et al., 2022). Few scientific reports have also indicated the superior potency of antioxidant properties in stingless bee honey (*Trigona sp)* as compared to manuka honey. Past research also revealed remarkable broad-spectrum activity against many Gram-positive and Gram-negative bacteria which are known for causing infections in DFU (Rasmussen et al., 2017). However, most of the studies related to stingless bee honey were conducted either in vitro or in vivo. Hence, clinical evidence on the efficacy of stingless bee honey in the treatment of wounds remains sparse (Biluca et al., 2020; Ooi et al., 2021; Ranneh et al., 2019). Moreover, given the prominence of medical-grade manuka honey and the lack of comparative studies between manuka honey and other types of honey in DFU, exploration of the latter remains elusive (Rao et al., 2016; Rosli et al., 2020; Yildiz Karadeniz & Kaplan Serin, 2023). In the local context, stingless bee honey produced by *Trigona sp* (also known as kelulut honey) is more commonly consumed and least explored for wound healing in human studies. Therefore, investigation into the potential of kelulut honey in the treatment of DFU is duly warranted for greater advantage to overcome the ever-growing antibiotic resistance and improve healing outcomes as the incidences of DFU complications are on the rise.

1.3 STATEMENT OF THE PROBLEM

DFU has become the primary concern of clinicians and patients globally since it is highly associated with morbidity, mortality, and high treatment costs (Hicks et al., 2020; Macdonald et al., 2018; Pemayun & Naibaho, 2017). The rising incidences of DFU are

inevitable since the prevalence of diabetes has hit a high note in the past few decades across the globe (Bus, van Netten, et al., 2016; Muldoon, 2013; Schmidt & Holmes, 2018). Despite the technological advancements in surgical interventions, the susceptibility of DFU to conventional methods and antibiotic resistance remains high. Thus, the resurgence of honey-based wound dressing is timely particularly in the challenging times of increasing infections, antibiotic resistance, gangrene, and diabetes-based lower limb amputations (Hixon et al., 2019). Although therapeutic properties and clinical efficacy of medical-grade manuka honey have been established, significant findings and high-quality clinical pieces of evidence in the treatment of DFU are lacking and remain inconclusive (Aina et al., 2018). Despite the potency of honey in eliminating infections and inflammations while improving healing, the therapeutic benefits of different types of honey were not optimised for the treatment of DFU.

With much preference given to medical-grade manuka dressing for wound healing, the potential of honey such as kelulut honey may have taken a back seat despite having similar nutritional values and therapeutic properties (Brown et al., 2020; Nordin et al., 2018; Ranneh et al., 2019). Laboratory-based in vitro findings that highlight the superior potential of antioxidant properties in kelulut honey have not been well explored to improve wound healing outcomes (Omar et al., 2019; Rao et al., 2016; Rashid et al., 2019; Saranraj & Sivasakthi, 2018). Hence, little knowledge is currently available on the efficacy of kelulut honey for the treatment of DFU as compared to the honey produced by *Apis mellifera*. Additionally, comparative studies between manuka honey and kelulut honey remain limited despite the urgency to pave the way for non-manuka honey-based wound dressing to improve healing outcomes in DFU, given its debilitating consequences.

Several studies have highlighted a number of risk factors associated with the healing of DFU which included patients' socio-demography, clinical characteristics, and ulcer characteristics. The majority of studies indicated the association of age, glycated hemoglobin A1c (HbA1c), site of ulcer, duration of ulcer, size of ulcer, vascular disease, and neuropathy with the healing rate of DFU (Lin et al., 2020; Mansoor & Modaweb, 2022; Yakoot et al., 2019; Zubair et al., 2019). Other studies highlighted self-management of diabetes, usage of advanced wound dressing, and infection management in influencing the healing outcomes of DFU (Deng et al., 2022; Musa & Ahmed, 2012; Rastogi et al., 2022;

van Netten et al., 2016; Vas et al., 2020). However, there is a lack of published data on the effect of socio-demographic and clinical characteristics in influencing the healing outcome of DFU when treated with honey. The efficacy of honey may not be optimised if the risk factors associated with the healing trajectory of DFU such as age, gender, HbA1c, body mass index (BMI), chronic kidney disease (CKD), duration of diabetes, and ulcer characteristics were not investigated even though they are crucial to formulate limb salvaging treatment strategies (Harries & Harding, 2015; Mavrogenis et al., 2018; Peter-Riesch, 2016). To date, exploration of the risk factors that influence the healing outcomes in DFU with honey-related studies remains limited (Purnama et al., 2021; Xu Tian et al., 2014; Wang et al., 2019).

Even though honey has been used for wound healing in clinical settings, significant evidence in the treatment of DFU remains limited. Based on previous studies, honey produced by honey bees (*Apis spp*), especially manuka honey, has shown promising wound-healing outcomes in DFU (Tian et al., 2014; Wang et al., 2019; Yildiz Karadeniz & Kaplan Serin, 2023). Despite the prominence of manuka honey-based wound dressing, there are forthcoming in vitro studies that revealed the potential of stingless bee honey in wound healing. Given the absence of clinical evidence regarding the efficacy of kelulut honey in wound healing, medical-grade manuka-honey-based wound dressing is primarily utilised for the healing of DFU in healthcare settings across the globe. To date, studies comparing the efficacy of manuka honey and stingless bee honey (kelulut honey) in the treatment of DFU are less common. Therefore, the present study could address the gap of knowledge on the effectiveness of kelulut honey compared to medical-grade manuka honey in DFU. Consequently, the associated factors influencing the healing outcome of DFU with kelulut honey could be evaluated.

1.4 RESEARCH OBJECTIVES

The main objective of this study is to evaluate the effectiveness of kelulut honey for the treatment of DFU. The specific objectives of this study are as follows:

1) To compare the effectiveness of kelulut honey and medical-grade manuka honey following MDT in the treatment of DFU based on the percentage of ulcer size reduction from baseline to day 7 and day 14.

2) To evaluate the relationship between the participants' socio-demography (age, gender, BMI), clinical characteristics (duration of diabetes, HbA1c, CKD), ulcer characteristics (grade, size, site, duration of ulcer), and the percentage of ulcer size reduction.

1.5 RESEARCH QUESTIONS

The following research questions were developed based on the specific objectives of the study to assist in the research design and methodology:

1) How is the efficacy of kelulut honey as compared to medical-grade manuka honey following MDT in the treatment of DFU based on the percentage of ulcer size reduction from baseline to day 7, and day 14?

2) Is there any relationship between the participants' socio-demography (age, gender, BMI), clinical characteristics (duration of diabetes, HbA1c, CKD), ulcer characteristics (grade, size, site, duration of ulcer), and the percentage of ulcer size reduction?

1.6 ALTERNATIVE HYPOTHESIS

Alternative Hypothesis 1, H_a1:

There is a significant difference between kelulut honey and medical-grade manuka honey following MDT in the treatment of DFU based on the percentage of ulcer size reduction from baseline to day 7 and day 14.

Alternative Hypothesis 2, H_a2:

There is a significant relationship between the participants' socio-demography (age, gender, BMI), clinical characteristics (duration of diabetes, HbA1c, CKD), ulcer characteristics (grade, size, site, duration of ulcer), and the percentage of ulcer size reduction.

1.7 SIGNIFICANCE OF THE STUDY

Despite advancements in the treatment of DFU, the alarming rise of preventable foot complications and lower-limb amputations among diabetics is becoming inevitable. The hard-to-heal DFU requires increased surgical interventions and prolonged hospitalisations, which could further escalate the financial burden on the public health of a developing country like Malaysia (Hopkins et al., 2015; Kee et al., 2019; Raghav et al., 2018). To a certain extent, the management of DFU has produced promising outcomes. However, the majority of diabetic patients still suffer from non-healing DFUs when surgical and conventional treatments fail to produce targeted healing outcomes. Honey-based wound dressings have been used as an alternative platform for clinicians to address the ever-growing antibiotic resistance and delayed healing of DFU in healthcare settings. The antibacterial, anti-inflammatory, and antioxidant properties of honey have largely contributed to the improvement of infected and complicated DFU (Angioi et al., 2021; Elbanna et al., 2014; Labban, 2014).

Several studies have demonstrated the potential use of medical-grade honey-based wound dressing as an alternative treatment when conventional treatment fails to heal wounds (Smaropoulos & Cremers, 2019; Xu Tian et al., 2014; Zabaiou et al., 2017). As significant clinical outcomes in the healing of DFU using medical-grade manuka honey are limited, the present study could provide much-needed clinical evidence for kelulut honey to be explored and utilised in the treatment of DFU. To date, there is a lack of comparison studies between different types of honey in the healing of DFU. Thus, exploring and comparing the efficacy of kelulut honey and medical-grade manuka honey in the treatment of DFU in this study can bridge the knowledge gap and widen the window of usage for kelulut honey from a superfood to a potential wound dressing to expedite the healing trajectory of DFU.

Besides appropriate wound dressing, the management of DFU would not be complete without effective wound bed preparation. Debridement in wound bed preparation is undoubtedly crucial for the acceleration of wound healing (Hingorani et al., 2016; Lim et al., 2017; Nuutila & Eriksson, 2021). Debridement has been the science behind wound bed preparation to remove slough and necrotic tissue to facilitate healing with other interventions or dressing. In this study, MDT using local species *Lucilia cuprina* was selected as the primary tool for debridement as MDT has been proven to debride, disinfect, and stimulate wound healing in DFU (Jordan et al., 2018; Nair et al., 2020; Nishijima et al., 2017). The latest findings on MDT in the local context further strengthen its significance for the healing of DFU. The efficacy of MDT as a potent debridement tool also stands as an added advantage to address the challenging and prolonged management of DFU (Manna et al., 2020; von Beckerath et al., 2020). Hence, MDT was used as the debridement method in this study. To the researcher's best knowledge, this study is the first that explores the effect of combining MDT with kelulut honey for the healing of DFU. Therefore, the present investigation acts as the pioneer research for the combination of MDT- kelulut honey in the treatment of DFU that could accommodate the healing trajectory of DFU.

1.8 DEFINITIONS OF KEY TERMS

Diabetes mellitus

Diabetes mellitus is a metabolic disease that is indicated by high levels of glucose in the bloodstream due to ineffective insulin or insufficient insulin (WHO, 2023).

Diabetic foot ulcer

Diabetic foot ulcer (DFU) is defined as a full-thickness wound developed among 25% of diabetics with poor glycemic control that takes a longer duration to heal. If remains untreated, DFU can lead to serious consequences such as infection, gangrene, and amputations (Lavery et al., 2016).

Honey

Honey is a sweet, viscous substance produced by bees through the accumulation of nectar from flowers. It has been shown to have healing properties when consumed or applied to wounds. The therapeutic properties include antibacterial, anti-inflammatory, and antioxidant properties (Münstedt, 2022)

Kelulut honey

Kelulut honey is a type of honey produced by stingless bees from the species of Trigona or Meliponine that has high antioxidant potency (Yaacob et al., 2017).

Medical-grade manuka honey dressing

Medical-grade manuka honey dressing refers to honey produced by honey bees from the *Apis mellifera* species, which has been sterilised and used for treating burns and wounds.

Methylglyoxal (MGO) is the main compound in this honey that is responsible for its antibacterial properties to combat infection in wounds (Hossain et al., 2023).

Maggot debridement therapy

The application of sterile maggots from *Lucilia cuprina* to de-slough wounds, disinfect, increase granulation, and accelerate healing (Abela, 2017)**.**

Clinical characteristics

Clinical characteristics are described as characteristics that define or are associated with certain diseases. HbA1c, duration of diabetes, and chronic kidney disease are among the clinical characteristics of patients with diabetes that could assist in the management of DFU (Monteiro-Soares et al., 2012)**.**

Ulcer characteristics

The characteristics of DFU include size, site, grade, and duration of ulceration. The size of ulcer refers to the area occupied by the ulcer that can be determined by manually or digitally calculating its length, width, and depth. The site of ulcer refers to the location of the DFU, such as the dorsum, lateral, plantar, and heel. The duration of ulcer also plays a vital role in the healing trajectory of DFU. Duration of DFU is defined as the time from patient-reported ulcers to the beginning of treatment initiation. The average projection of DFU healing is 4 to 12 weeks. The longer the ulcer exists, the time taken to heal also tends to be extended. Many types of DFU classification describe the severity of DFU and define it as the grade of ulcer. The validated, commonly used grading system of DFU includes the Wagner classification system, compassing grades 0-5 that define the ulcer as having no open lesion (grade 0), superficial (grade 1), full-thickness wound (grade 2), full-thickness wound with infection and abscess (grade 3). On the other hand, Grades 4 and 5 indicate gangrene with severe infection that may require amputation of toes or limbs (Fife et al., 2016; Patry et al., 2021; Rossboth et al., 2021).

Socio-demography

Socio-demography refers to a combination of social and demographic factors (e.g., age and gender) that define individuals in a population (Vo et al., 2023)**.**

1.9 STRUCTURE OF THE THESIS

This thesis is divided into 6 chapters:

Chapter 1

The first chapter introduces the research background, problem statement, research objectives, and research questions. It also outlines the significance of this study, definitions of key terms, and the thesis structure.

Chapter 2

This chapter encompasses four segments with the first segment discussing DFU, wound healing process, and DFU management. The second section contains details regarding wound dressings and honey dressing, followed by a discussion about the potential efficacy of stingless bee kelulut honey in wound healing in the third section. The fourth section presents the literature search strategy and findings that justify the relevance of this study.

Chapter 3

This chapter describes the research design as well as the methodology and instruments used to conduct this study. It also illustrates the ethical considerations, proposed statistical analysis, and data collection process.

Chapter 4

This chapter presents the results of the present study, which would be obtained through statistical analysis done via IBM SPSS version 29. It also discusses the baseline

characteristics of the participants and the normality of data distribution. The main highlight of this chapter is the outcome concerning the percentage of ulcer size reduction that defines the efficacy of honey in DFU. This chapter also reports the correlation between sociodemographic factors, clinical characteristics, ulcer characteristics, and the percentage of ulcer size reduction.

Chapter 5

This chapter discusses the findings presented in Chapter 4, including the statistical analysis results that determine the differences in efficacy between both kelulut honey and medical-grade manuka honey based on the percentage of ulcer size reduction. It also discusses the findings on the associated factors related to the healing of DFU.

Chapter 6

This chapter highlights the strengths, limitations, novelty, and implications of this study along with several recommendations for future research. It also discusses the relationship between the percentage of ulcer size reduction and socio-demographic factors, clinical characteristics, and ulcer characteristics.

1.10 CHAPTER SUMMARY

Wound healing outcomes of DFU are highly dependent on the utilisation of an effective and appropriate wound dressing to enhance the healing process. As DFU takes a longer time to heal with conventional methods, the utilisation of honey-based dressings has played a substantial role in producing promising healing outcomes for DFU in the healthcare setting (Mavrogenis et al., 2018; Yildiz Karadeniz & Kaplan Serin, 2023). Despite the prominence of medical-grade manuka honey dressing, the lack of significant clinical findings in the treatment of DFU has created a limited window of usage for honey in the healthcare setting. While kelulut honey has demonstrated beneficial physiochemical properties that could be a potential for wound healing, its usage and implications have been less explored. Therefore, the present study was undertaken to establish the much-needed

evidence base for kelulut honey as a potential wound dressing in the treatment of DFU in comparison to medical-grade manuka honey. Furthermore, the combination of MDT-honey may accelerate the healing outcomes for DFU, which is considered the first to date. Consequently, kelulut honey can be utilised to the best of medical advantage to improve the healing trajectory of DFU and reduce diabetes-based foot complications.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW

The role of honey in wound healing has been promising and undeniably useful in the treatment of DFU. To understand the efficacy of honey in wound healing, this chapter presents a brief introduction to DFU, followed by the wound healing process and management of DFU. The second section discusses conventional wound dressings and the use of honey in the treatment of DFU. The third section emphasizes the potential efficacy of stingless bee kelulut honey from the medicinal perspective to enhance healing outcomes. The fourth section presents the literature search strategy to find relevant studies and discussions in the existing literature by focusing on comparative studies between honey and other dressings or therapies in the treatment of DFU. Finally, this chapter concludes with a summary and conclusion of the literature review.

2.2 DIABETIC FOOT ULCER

As the prevalence of non-communicable diseases like diabetes mellitus increases, incidences of diabetes-based foot complications are also on the rise. Clinical findings indicated that one of the worst, most devastating complications of diabetes is foot ulceration (Jain & Barman, 2017). Diabetic foot ulceration, commonly known as DFU, is a full-thickness skin loss occurring in poorly controlled diabetics, which could lead to serious consequences if remain untreated. Delays in wound healing and recurrences of DFU have caused much distress to diabetic patients who lose mobility and quality of life. In a larger

context, DFU has been identified as a threat to public health and a financial burden to developing countries (IDF, 2021).

As the resistance of DFU towards conventional methods increases, it has been proven to be a strong predictor and precursor to about 80% of lower-limb amputations in many parts of the world (Pickwell et al., 2015; Schmidt & Holmes, 2018; Thewjitcharoen et al., 2014). It was estimated that 25% of poorly controlled diabetics would develop DFU in their lifetime, which mainly occurs due to peripheral neuropathy, poor vascularity, and poorly controlled diabetes. Peripheral neuropathy in diabetics causes loss of sensation, which makes it difficult for the patients to realize any changes in the skin or injuries on the foot for immediate responses (Raghav et al., 2018). Furthermore, DFU worsens as hyperglycemia causes damage to the blood vessels, leading to poor blood flow to the feet. Hyperglycemia also impairs the body's immune response and causes the slow healing of DFU. Furthermore, DFU is categorised as a chronic wound since it takes a longer period to heal with conventional methods (Dugbartey et al., 2022). Complications of DFU include infection, gangrene, and lower limb amputations. The majority of DFUs fail to follow the normal wound healing process, which consequently leads to serious foot complications such as lower limb amputations (Jeffcoate et al., 2018).

2.3 CHRONIC WOUND HEALING PROCESS

Chronic wounds generally include venous ulcers, DFU, or pressure ulcers which commonly show great resistance towards conventional methods over the years. According to Hinchliffe et al. (2016), DFU that develops below the ankle irrespective of duration in a diabetic patient can turn chronic fast and is hard to heal. With the progression of peripheral neuropathy, the majority of patients showed no symptoms until the wound became chronic, which took longer than 3 months to heal. Thus, DFU goes through the devastating non-healing phase. Management of DFU remains a challenge for many clinicians for decades and causes a debilitating impact on patients' quality of life (Kiliçoglu et al., 2018; Pemayun et al., 2015).

In general, chronic wounds do not follow the normal healing process of acute wounds. As mentioned in the guidelines for the management of foot disease, an acute wound goes through the normal process of healing, which includes hemostasis, inflammation, proliferation, and maturation. However, an acute wound may transform into a chronic wound due to underlying pathological conditions such as diabetes, infection, vascular insufficiency, and peripheral neuropathy (Lipsky et al., 2016; Macdonald et al., 2018; Vowden & Vowden, 2014). Generally, the time taken for acute wounds to heal tends to be shorter than chronic wounds as it gets stuck in the inflammatory stage and fails to heal over the normal healing phase of 1 to 3 months. According to Wallace et al. (2023), the general wound healing process starts with hemostasis, which is recognised as the first step of response to traumatic injury that involves vasoconstriction, blood clotting, platelet aggregation, and fibrin formation. It is followed by the inflammation phase (0-4 days), which includes neutrophil, lymphocyte, monocyte infiltration, differentiation to macrophages, and ultimately activates vasodilation. This process induces blood flow causing heat, redness, pain, swelling, and loss of function. Additionally, wound exudate may present at this stage. The proliferation phase (2-24 days) will happen after the inflammation phase where tissue repair, granulation, epithelialisation, collagen synthesis, and extracellular matrix formation will take place. Reduction in the wound size is to be observed at this phase. The maturation phase (24 days-1 year) is the final stage of the wound healing process which occurs with the remodeling of collagen, vascular maturation and regression, and formation of scar tissue (Nazarko, 2015).

According to Yazdanpanah et al. (2015), chronic wounds can only achieve successful healing if the process is duly followed in a proper sequence and time frame. DFU tends to exhibit impaired healing and progresses into pathological inflammation caused by a non-systematic healing process. Few studies have indicated prolonged inflammation, persistent infections, and drug-resistant biofilms as inhibiting factors in the healing of DFU (Assaad-Khalil et al., 2015; Banu et al., 2015; Gomes et al., 2017; Guest et al., 2018). Infections in DFU play a predominant role in foot complications and become a potential risk for lower limb amputations. In a recent study, Shaheen et al. (2022) revealed diabetic patients with non-healing foot ulcerations may succumb to severe infection, osteomyelitis, gangrene, and lower limb amputation. Therefore, managing DFU would include the aspect

of infection control, wound debridement, the selection of appropriate wound dressing, and possible surgical intervention to improve healing outcomes (Singh et al., 2017).

2.4 INFECTION IN DFU

Various pathogens have been documented to cause infections in DFU and are often dominated by polymicrobial and multidrug-resistant organisms. Infection in DFU has been known to hamper the efficacy of common antibiotics and delay healing, given the fact that multi-drug organisms tend to form extracellular protective biofilms that are resistant to common antibiotics (Clokie et al., 2017). The most common pathogens infecting DFUs are Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis*, and Gram-negative bacteria including *Escherichia coli, Klebsiella pneumonia, Proteus spp*, and *Pseudomonas aeruginosa* (Jain & Barman, 2017). However, several reports have indicated that pathogens differ according to locality whereby pathogens in Asia and other developing countries are shown to have more gram-negative pathogens. Nevertheless, the findings remain argumentatively academic. *Staph aureus* is the most isolated in DFUs and prevalent either in developed or developing countries (Essebe et al., 2017).

Based on a consensus recommendation by the Infectious Disease Society of America (IDSA), the treatment of infected DFU must be aggressive upon diagnosis to prevent foot complications. The report clearly states that empirical antimicrobial treatment should be initiated until the causative pathogens and their antibiotic susceptibility are "known" (IDSA, 2020). Given the current recommendations and past experiences, management of infected DFU includes anti-microbial therapies, surgical intervention, and debridement. Current antibiotics used in healthcare settings to counter infection in DFU include vancomycin, which is the most effective in treating Gram-positive bacterial infections including the treatment of multi-resistant *Staphylococcus aureus.* Other effective choices of antibiotics include cephalexin, dicloxacillin, amoxicillin-clavulanate, or clindamycin (Abd Wahab et al., 2015; Kwon & Armstrong, 2018). However, infection in DFU is rarely limited to one pathogen and this gives rise to the usage of different types of

topical and systemic agents in the management of infected wounds (Banu et al., 2015; Doğruel et al., 2022; Noor et al., 2017; Rahim et al., 2017).

Several studies have indicated that the utilisation of broad-spectrum antibiotics over time has inadvertently created an overgrowth of resistant bacteria. Consequently, there is an increase in antibiotic-resistant organisms leading to prolonged management of DFU and high treatment costs (Barwell et al., 2017; Hopkins et al., 2015). Antimicrobial resistance has been reported as one of the top ten threats to global health. Such condition happens when microorganisms no longer respond to antibiotics to which they are susceptible previously (Schaper et al., 2020). The phenomenon of antibiotic resistance has become a predominant risk factor for the healing of DFU for the past few decades. Consequently, infections become difficult to treat and at times impossible, which increases the risk of sepsis and even mortality among patients with non-healing infected DFU (Mancuso et al., 2021).

To strike a balance between administering suitable antibiotics and preventing the use of broad-spectrum antibiotics, clinicians uncovered more pharmaceutical advances in the form of topical antibiotics or dressings (Piperaki et al., 2019; Zubair, 2020). Based on the Cochrane systematic review, topical antimicrobial dressings are more efficient in inhibiting bacterial growth than non-antimicrobial dressings in the management of infected DFU. Apart from pharmaceutical advancements and interventions, effective wound bed preparation via debridement has also shown positive outcomes in reducing bioburden in DFU (Dumville, 2017; Falanga, 2018; Hingorani et al., 2016). Concerning the present study, MDT was used as the mode of debridement to prepare the wound for further treatment. The following section contains a brief introduction to debridement and the role of MDT.

2.5 WOUND DEBRIDEMENT

International guidelines by IWGDF on the management of DFU stress the importance of wound bed preparation via effective debridement. The objective of wound debridement is

to create an optimal healing environment for chronic wounds to granulate, epithelise, and heal at a faster rate (Bazaliński et al., 2019). The wound bed preparation framework known as T.I.M.E highlights the aspect of debridement to assist clinicians in expediting the healing of chronic wounds. The abbreviation for T is tissue evaluation, I for infection control, M for moisture balance and E for edge of wound (Game, Attinger, et al., 2016; Leaper et al., 2012; Panuncialman & Falanga, 2009; Sibbald et al., 2011). The principles in the T.I.M.E framework are summarised in Table 1.

Table 1: T.I.M.E Framework

T = Tissue	**I = Infection Control**	**M = Moisture Balance**	**E = Edge of Wound**
Evaluate if there is non-viable tissue, slough, eschar, or necrosis/biofilms on the wound bed. Select an appropriate debridement method to remove non-viable tissue, slough, eschar, or necrosis/biofilms.	Assess the presence of local or systemic infection, edema, and inflammation which can create a barrier to healing. Select appropriate local or empiric antibiotics to manage infection.	Determine if the wound is too dry/wet. The concept of moist wound healing is to strike a balance in moisture control. Select a suitable dressing.	Assess the edge of wounds; advancing or undermined? If the wound is healing, edges may appear migrating, open, or contracting. If non-suitable dressings are applied, the edge becomes undermined or rolled, especially in tunneling wounds.

Based on the T.I.M.E framework, the importance of debridement should not be underestimated in wound bed preparation. Hence, debridement becomes a focal point in removing non-viable tissue and preparing the wound for further intervention within the process of wound healing in DFU (Simman & Hermans, 2017). It is crucial for the non-viable tissue, such as slough, to be removed in the management of DFU as it can become a "nutrient source" for bacteria to multiply and possibly create a biofilm. Moreover, the presence of non-viable tissue can also act as a barrier to the topical application of wound healing materials to penetrate and produce their potency (Chiwanga & Njelekela, 2015; Everett & Mathioudakis, 2018; Kavitha et al., 2014; Lauerman et al., 2018). The presence of slough and necrotic tissue can prevent granulation and epithelialisation, which hampers normal extracellular matrix formation. More importantly, non-viable tissue could also mask the presence of bioburden on the wound bed (Manna et al., 2020). Therefore, the need to employ the most appropriate debridement tool to optimise wound bed preparation and remove healing barriers is highly crucial (Aumiller & Dollahite, 2015; Jordan et al., 2018).

An efficient debridement process can convert a chronic wound to an acute profile and consequently, subsequently jump-starting the wound towards a healing trajectory in the management of DFU. Various conventional debridement methods have been in practice since the 19th century, including surgical and non-surgical methods. Boulton et al. (2018) pointed out the importance of surgical debridement, especially in limb-threatening infections with gangrene or necrotic eschar. To date, surgical debridement has been recognised as the gold standard in achieving fast debridement outcomes. Nevertheless, non-surgical methods should not be underestimated as the clinical evidence for other conventional non-surgical methods using mechanical, autolytic, and enzymatic methods is quite promising (Elder & Grover, 2013; Lumbers, 2018). According to Nishijima et al. (2017), excessive bleeding, non-selectivity, and requirement for local or general anesthesia are the drawbacks of most conventional debridement methods in clinical settings. Therefore, it is concluded that conventional debridement methods may not be applicable for all wounds to remove healing barriers in wound bed preparation (Sorg et al., 2017). Considering debridement as the science behind wound bed optimisation, failing to do so will delay the healing process. Biological debridement using MDT has shown promising and significant outcomes in the debridement of DFU compared to the non-surgical

conventional method (Daeschlein et al., 2017; Linger et al., 2016; Marimuthu & Makhtar, 2020). Hence, MDT was utilised in the present study to debride the sloughy DFUs based on previous findings that show significant outcomes in the treatment of DFU using *Lucilia cuprina*.

MDT is defined as using sterile maggots of *Lucilia spp*, green bottle-fly to remove slough and devitalise tissues from chronic wounds through the mechanical action of the maggots. The method has shown promising outcomes in the treatment of DFU despite the increasing trend in antibiotic resistance and futile conventional treatments (Nair et al., 2020; von Beckerath et al., 2020). The history of MDT goes back to 1931 when Dr. Baer used maggots to treat osteomyelitis among children in the United States of America. MDT was approved by the Food and Drug Administration (FDA) in 2004 as a medical device for the treatment of chronic wounds. Consequently, it gained the attention of clinicians across the globe to explore the therapy in chronic wounds using maggots by *Lucilia sericata* (temperate species) and *Lucilia cuprina* in the local context. The rationale behind the utilisation of MDT is that the sterile maggots of *Lucilia spp* are selective and only consume slough and devitalised tissue, leaving the healthy tissues intact. Moreover, clinical studies have shown the debridement strength of *Lucilia sericata* where 100 free-range sterile maggots were able to ingest 50 mg of slough/devitalised tissue (Choudhary et al., 2016; Nasoori & Hoomand, 2017; Shi & Shofler, 2014). Various studies have displayed the efficacy of MDT in improving wound healing outcomes due to its mechanism of action which breaks down slough, necrotic, and non-viable tissue, ultimately disinfects and stimulates granulation (Abela, 2017; King, 2020). Despite some reservations from clinicians and patients, MDT has stood the test of time and has been used effectively in the treatment of DFU as the last option for salvaging limbs (Naik & Harding, 2017; von Beckerath et al., 2020).

In the local context, similar findings have been reported concerning the use of *Lucilia cuprina* for the debridement of DFU (Azad et al., 2016; Marimuthu & Makhtar, 2020; Nair et al., 2020; Paul et al., 2009). Numerous studies have advocated the undeniable efficacy of MDT for debridement in the management of DFU. The sterile maggots de-slough the wound by secreting proteolytic enzymes and mechanically loosening the slough,

thus dissolving devitalised tissue effectively. The disinfection mode takes place as the maggots' secretions release ammonia which eventually increases the pH of the wound bed and inhibits bacterial growth and breakdown of biofilm. The secretion and excretion contain growth-stimulating factors that increase fibroblast proliferation and tissue regeneration. The mode of action for MDT consists of 3 main mechanisms, namely debridement, disinfection, and healing stimulation (Atamna & Elis, 2017; L & Aradhyamath, 2017; Manna et al., 2020; Sun et al., 2014). The mechanism of action for MDT is illustrated in Figure 2.

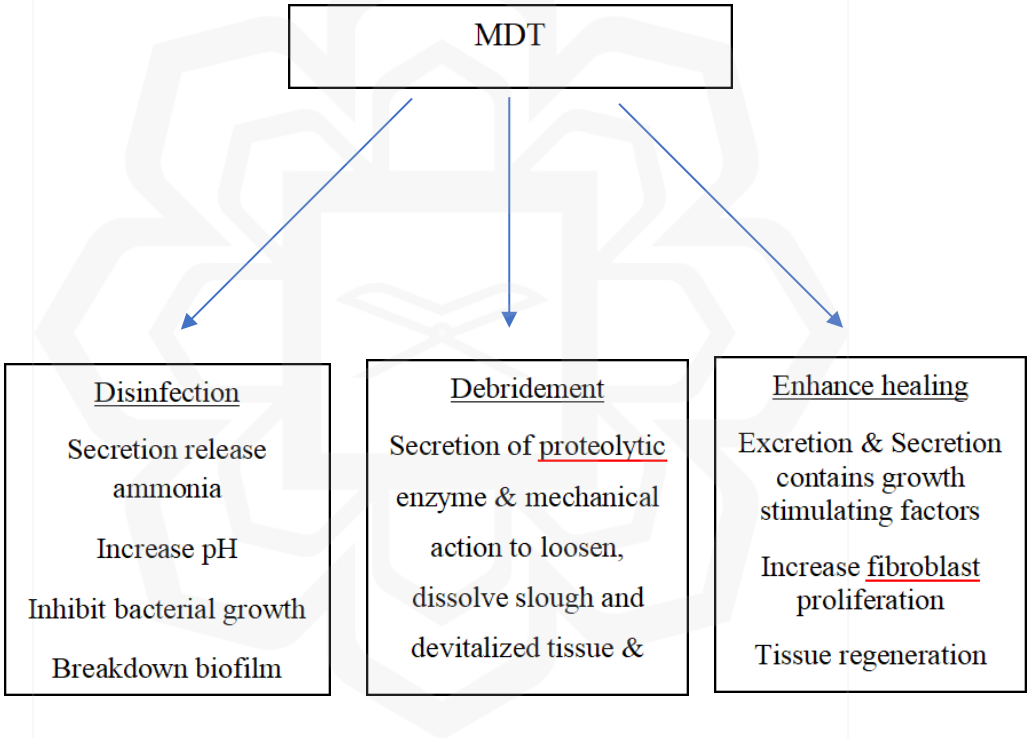

Figure 2: MDT Mechanism of Action

The successful debridement by MDT complemented with appropriate dressing sets the foundation for placing chronic wounds such as DFU in the healing trajectory. The next section pursues the current trends in wound dressing.

2.6 WOUND DRESSING

Wound dressing has undoubtedly gone through an evolutionary paradigm shift from the mere usage of gauze and cotton to advanced materials focusing on moist wound healing. According to Kateel et al. (2016), various types of topical wound dressing are available for the management of chronic wounds, which form a base of contact with the wound to stimulate healing. More importantly, topical wound dressing should be wound-specific, provide moist wound healing, allow gas exchange between the wound and environment, maintain tissue temperature, be non-adherent to the wound, safe and non-toxic, inhibit bacterial activity, effective exudate management, promote angiogenesis, and enhance epidermal migration. Selecting appropriate wound dressing is crucial to determine the success or failure of the wound healing process (Macdonald et al., 2018; Rezvani et al., 2019; Vowden & Vowden, 2014).

From the historical perspective, Bredow et al. (2018) postulated that conventional methods using saline-moistened gauze were predominantly utilised as standard dressing before the 1980s in almost all healthcare settings worldwide. As wounds became more complicated, modern dressings such as povidone gel, hydrocolloids, and polyurethane foams were introduced in the mid-80s to achieve moisture balance, inhibit bacteria, and exudate management (Kiliçoglu et al., 2018). When incidences of non-healing DFU increased, the evolution of wound dressing has brought advanced dressing such as silver alginate dressing, collagen dressing, hyaluronic acid, hydrogels, synthetic foam dressing, and vapor-permeable adhesive films to the forefront of chronic wound treatment in the mid-90s (Mavrogenis et al., 2018). However, very few wound dressings have prospective data to support their efficacy in enhancing wound healing. Several types of conventional and advanced wound dressings were also reported to be ineffective in inhibiting bacterial growth, and managing exudates and ultimately were unable to enhance granulation and shorten healing time. Hence, the choice of topical antimicrobial dressing stays limited to povidone iodine-based dressing, silver alginate, and antibiotic creams in many healthcare settings (Kus & Ruiz, 2020) .

The selection of appropriate wound dressing has become one of the major elements for efficient wound management for DFU in recent times. Looking back into the past, natural-based biological alternatives have been explored for wound healing in the form of antimicrobial wound dressing to optimise healing outcomes (N. 'Izzah Ibrahim et al., 2018). Technological advancements in the field of wound care have brought honey-based dressing to the limelight to improve healing outcomes and prevent foot complications. To date, several studies have highlighted the efficacy of honey dressings in providing a moist wound-healing perspective with added therapeutic efficacies to accelerate wound healing (Al-Hatamleh et al., 2020; Wang et al., 2019).

2.7 BACKGROUND OF HONEY

Honey is one of the first natural sweeteners discovered by man produced by honey bees *Apis mellifera*. The historical perspective of honey dated back to the *Paleolithic* era and there was also documentation of bee-keeping activities based on cave paintings in Spain, dating back to the Neolithic era (Yilmaz & Aygin, 2020). Evidence for the early usage of honey was based on the clay tablet found by scientists dating back to 2100-2000 years BC. More findings based on an artifact found in Egypt's tombs indicated honey as a medicinal and ceremonial substance (Fratellone et al., 2016; Zabaiou et al., 2017). Beekeeping activities were also evidently present in other parts of the world (e.g., Greece and Rome) where honey was consumed for energy and treatment of illnesses. Traditional medicine using honey also played a big role, especially in India where it was widespread in Ayurvedic treatment (Henshaw et al., 2014; Mandal & Mandal, 2011; Samarghandian et al., 2017; Ulasan et al., 2020). Thus, honey is not new and rediscovered for modern medicinal use.

For centuries, the distribution of evidence on the usage and relevance of honey was found to be crossing borders mainly for consumption to improve one's health. Therefore, the demand for honey increased mainly due to its recognition as a potential health supplement (Nolan et al., 2019). However, honey has received much recognition for its

medicinal properties based on the scientific data accumulated around the globe in the past two decades. With the availability of honey worldwide, researchers looked into the efficacy of honey and other products of bees for pathological treatments and initiated the term "apitherapy" (Badolato et al., 2016; Trumbeckaitė, 2014). *Apis* means bee in Latin and Apitherapy is defined as treatment using bees or bee products such as pollen, propolis, honey, royal jelly, and even bee venom to improve human health (Badolato et al., 2017). In ancient times, apitherapy was used as a traditional remedy or medicine for the treatment of disease with bee products in many parts of the world. Previous evidence has shown apitherapy as part of ancient Greek, Egyptian, Roman, and even Chinese traditions, dating back to more than 6000 years ago (Zabaiou et al., 2017).

One of the first scientific research on apitherapy was conducted more than 50 years ago involving the use of royal jelly for the treatment of heart disease, followed by an evaluation of propolis as a supplement for maintaining good health in Germany (Siavash et al., 2015). Since apitherapy shows almost no side effects, it has received much attention mainly for therapeutic purposes. Initial findings demonstrated promising beneficial outcomes of apitherapy in reducing infections and inflammation while boosting immunity. Further investigations into the therapeutic properties of apitherapy revealed considerable potential medicinal value and were incorporated into modern medicine for treating chronic diseases (Brown et al., 2020). Thus, apitherapy was included in the WHO Traditional Medicine Strategy (2014–2023) as an alternative for disease management and prevention such as cough, tuberculosis, and burns. However, among all products of apitherapy, honey was specifically mentioned in this report as a potential nutritional and health supplement (WHO, 2015).

2.7.1 Therapeutic Properties of Honey

According to Yilmaz & Aygin (2020), scientific evidence on the efficacy of honey in modern medicine remained anecdotal until 1999. However, the earliest research on the modern approach to honey was convincingly initiated in 2000 as studies on the therapeutic properties of honey started to emerge. Thus, honey's window of usage evolved and

expanded from a superfood to a healing agent based on clinical evidence suggesting potential therapeutic efficacies of honey (Huanbutta et al., 2020). Generally, honey encompasses a complex mix of carbohydrates (84%), water (17.1%), as well as proteins, amino acids, vitamins, phenolic compounds, and organic acids (0.5%). However, the composition of honey is highly dependent on the geographical aspect, weather conditions, and type of flowers (either unifloral or polyfloral) visited by the honeybees (Kateel et al., 2016; Siraj et al., 2018). For example, the composition of honey produced in Europe may be slightly different than those in New Zealand or Asia depending on the botanical aspect of the honeybees. The therapeutic properties of honey may show more similarities than differences despite being produced in different regions (Santos-Buelga et al., 2017). However, the origin of honey revolved around the honey bees *Apis spp,* which has been proven to be an effective therapeutic agent since ancient times. Honey produced by the honey bees *Apis mellifera* is recognised as one of the oldest medicines in disease prevention, especially as an antibacterial agent to fight infections (Toledo-Hernández et al., 2022).

Boosting immunity against infection is the main reason why honey is consumed as a superfood worldwide, with past research suggesting it as a potential therapeutic agent for the management of chronic conditions (Mărgăoan et al., 2021). Considerable evidence on the successful therapeutic utilisation of honey in general and medical conditions has been reported in recent times. Recent studies indicated that the presence of a small percentage of phenolic compounds in honey is highly associated with anti-oxidant and anti-inflammatory properties. As highlighted by Zeleníková and Vyhlídalová (2019), the physiochemical of honey varies depending on the types of vegetation from which the bee consumes nectar. Nevertheless, there are similarities between phenolic acids in different types of honey, including caffeic, ellagic, ferulic, and p-coumaric acids; flavonoids, such as apigenin, chrysin, galangin, hesperetin, kaempferol, pinocembrin, quercetin; and antioxidants, such as tocopherols, ascorbic acid, superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Each of the phenolic compounds was reported to have unique nutritional and medicinal properties. These compounds act synergistically, lending honey utility in a variety of applications (Cianciosi et al., 2018; Juszczak et al., 2016). Hence, the physical properties and chemical composition of honey fluctuated based

on the plants from which the bees collect raw material. Moreover, differences in the type of flora, climatic conditions, and geographical region also influence honey's physical and chemical properties (Raja Nurfatin et al., 2021). Based on laboratory-based investigations and clinical studies, honey has shown promising therapeutic benefits in several major disease management such as diabetes, cancer, gastrointestinal, vascular, neurological disorders, antihyperlipidemic disease, and wound care due to its antibacterial, anti-inflammatory, and antioxidant capacity (Biglari et al., 2012; Ewnetu et al., 2013; Kumar Gupta & Stangaciu, 2014; Lima et al., 2020; Mandal & Mandal, 2011; Mărgăoan et al., 2021; Rao et al., 2016).

2.7.2 Types of Honey

There are various types of honey across the globe. However, the majority of honey production is from honey bees such as manuka honey while the least known is those produced by stingless bees (Omar et al., 2019). The physiochemical properties of honey are highly dependent on its floral source and are often named based on its geographical location or source of the plant visited by bees. Honey is categorised based on its origin and the most promising type of honey for medicinal use came from monofloral honey (Alvarez-Suarez et al., 2018). As mentioned, honey bees of *Apis mellifera* origin are the most common of the seven species of honey bees across the globe, which are commonly known as western honey bees or European honey bees. The well-known manuka honey is a monofloral honey produced by honey bees *Apis mellifera* and obtained its name from a wildflower known as manuka (*Leptospermum scoparium*) which grows in the forests, hills, and coastal areas in Australia and New Zealand (McLoone et al., 2016; McLoone et al., 2020). The honeycomb or hive and honey bees that produce manuka honey *Apis mellifera* are shown in Figures 3 and 4, respectively (adapted from Rao et al., 2016).

Figure 3: Hive of honey bee *Apis mellifera*

Figure 4: Honey bee *Apis mellifera*

Manuka honey has a rich concentration of methylglyoxal (MGO)**,** a unique compound that is cytotoxic and proven to have an inhibitory effect on 60 species of bacteria including aerobes and anaerobes (Martini, 2016). Studies have revealed that high MGO is

responsible for the major antibacterial effect of manuka honey in wound healing (Carter et al., 2016). Wound dressing using manuka honey has been highlighted as a non-antibiotic treatment. Medical-grade manuka honey was presumed as a much-awaited solution in the battle against *methicillin-resistant S. aureus* in the management of DFUs (Frydman et al., 2020; Zeleníková & Vyhlídalová, 2019). Given the promising outcomes, manuka honey has become the center point for medical research and the most commercially produced honey in the medical field worldwide (Gill et al., 2019; Tsang et al., 2015). In the field of wound care, medical-grade manuka honey of *Apis mellifera* has been incorporated into topical wound dressing in the form of gel, cream, and alginates. Medical-grade manuka honey has been reported to cause less reactions in the human system due to sterilisation using Gamma irradiation. Numerous studies have highlighted the beneficial anti-bacterial properties of medical-grade manuka honey in the treatment of partial-thickness burns, infected and post-operative wounds, as well as sloughy, necrotic, and smelly wounds (Cooper, 2014; Nikpour et al., 2014; Oryan et alo., 2016). However, significant efficacies of medical-grade manuka honey in DFU have only been concluded in a limited number of high-quality clinical studies. The acidic nature of manuka honey, high osmotic pressure, and the presence of MGO were reported in majority of past studies as the science behind the anti-bacterial properties of medical-grade manuka honey (Fratellone et al., 2016; Girma et al., 2019; Müller et al., 2013).

Based on the laboratory analysis, MGO is a protein-glycating agent responsible for the non-peroxide activity observed in honey. Despite MGO being a factor in the anti-bacterial properties of medical-grade manuka honey, several isolated contradicting reports stated that MGO may inhibit wound healing in DFU (Martinotti et al., 2018). It raises a question on the safety of MGO's action in the wound healing of DFU as the production of advanced glycation end products was reported to be one of the causes of health complications in diabetes (Hossain et al., 2023; Jull et al., 2015; Valachova et al., 2014). Nevertheless, medical-grade manuka has captured the limelight in modern wound care and shown great potential in tissue repair and regeneration in the treatment of infected wounds like DFU. As mentioned in previous studies, a high concentration of MGO was associated with the inhibition of bacteria namely methicillin-resistant *Staphylococcus aureus* (MRSA)

and vancomycin-resistant Enterococci (VRE). MGO has been proven to remove MRSA from colonised wounds and inhibit MRSA via disrupting cell division (Eick et al., 2014; Müller et al., 2013). Therefore, further investigation on the MGO compound in manuka honey is crucial due to inconsistent findings on the effect of MGO.

In a local public healthcare setting, the infection rate in DFU was reportedly as high as 41% in Malaysia (Kee et al., 2019). Thus, it is understandable why a wide interest has been shown in medical-grade manuka honey. Despite the high potency of medical-grade manuka honey, existing clinical studies are more prominent in countries such as New Zealand, Australia, the United Kingdom, Pakistan, and India. This is due to the presence of the manuka plant, the abundance of honey produced by *Apis spp,* and economically-based beekeeping activities as opposed to other regions. In Malaysia, Tualang honey from *Apis dorsata* has gained prominent research interests while the least explored in the clinical field is honey produced by stingless bees (Khalil et al., 2011; Siavash et al., 2015; Xu Tian et al., 2014; Zainol et al., 2013).

2.7.3 Role of Honey in Wound Healing

The renewed interest in honey for the healing of chronic wounds was spurred by the increasing number of foot complications where conventional methods failed to produce targeted outcomes and overcome antibiotic resistance. Inhibition of bacterial growth without causing antibiotic resistance demonstrated by honey is one of the main contributory factors for honey to be considered as a wound dressing in chronic wounds such as DFU to improve healing outcomes (Astrada et al., 2019). Laboratory-based investigations have revealed antimicrobial and anti-oxidant properties of honey as the focal point for it to be incorporated into modern wound care in many parts of the world since the 19th century (Gill et al., 2019; Meo et al., 2017; Ulasan et al., 2020). For medical purposes, honey was sterilised to eliminate contamination by *Clostridium botulinum* spores, and eventually the term “medical-grade honey” emerged. The benefits of honey in wound healing are further enhanced by the osmotic effect of honey to draw fluid from the wound bed and dehydrate bacteria. Moreover, the anti-inflammatory effect reduces swelling and pain and increases

blood flow to the wound site (Al-Hatamleh et al., 2020; Naik et al., 2021; Vyhlídalová et al., 2018).

Based on previous studies, the therapeutic properties of honey can be categorised into five main efficacies that could contribute to wound healing (Denisow & Denisow-Pietrzyk, 2016; Elbanna et al., 2014; Hussain et al., 2015; Lima et al., 2020; Oryan et al., 2016; Zabaiou et al., 2017). Firstly, the antibacterial activity of honey is contributed by antioxidants in stingless bee honey (*Trigona sp, Meliponii sp*) and MGO in honey bee *Apis sp*. Secondly, the debridement effect is due to the inhibition of tissue plasminogen activator which eventually reduces the accumulation of slough and non-viable tissue on the wound bed. Thirdly is the removal of free radicals by the antioxidants in all honey which contributes to its anti-inflammatory effect. Fourthly, the osmotic effect provides the much-needed moist environment that is appropriate for healing of wounds. Lastly, the healing of wounds is further stimulated by the release of oxygen through the acidification process in all honey which enhances granulation and eventually promotes accelerated healing (Cooper, 2014; Mărgăoan et al., 2021; Meo et al., 2017; Rao et al., 2016). The therapeutic efficacy and its mechanism of action are summarised in Table 2

Table 2: Therapeutic Properties of Honey in Wound Healing

Therapeutic Efficacy	Mechanism of Action
Antibacterial activity	Antimicrobial factors (either hydrogen peroxide or MGO)
Debridement effect	Inhibition of tissue plasminogen activator inhibitor
Anti-inflammatory	Quenching of free radicals by anti-oxidants; modulation of cytokines
Provide a moist wound-healing environment	Osmotic effect
Enhance healing	Acidification of wound promotes the release of oxygen, hastens granulation

Considerable evidence from case studies and clinical controlled trials have indicated the promising outcome of honey in reducing wound infection odor while stimulating healing in the treatment of burns, soft tissue injury, and chronic wounds. This approach is crucial to address the issue of antibiotic resistance, infection, exudate management, and stimulate healing with the least pain and side effects (Banu et al., 2015; Karri et al., 2016). To date, medical-grade honey is reported to have excellent anti-bacterial activity and there is no evidence to show the existence of honey-resistant phenotypes. Considering the upward trend of antibiotic resistance and the chronicity of DFU, the potential of honey to become an effective wound dressing is inevitably useful and undeniably advantageous in resolving the rapidly increasing complications of DFU (Müller et al., 2013; Nishio et al., 2016; Smaropoulos & Cremers, 2019).

In general, honey produced by both honey bees and stingless bees possess similar antibacterial properties to inhibit bacterial growth and kill pathogens (Esa et al., 2022). However, Cianciosi et al. (2018) asserted that the difference lies in the absence of MGO in honey produced by stingless bees, which is responsible for the antibacterial activities of honey bees. The MGO compound in manuka honey stimulates cytokines to induce an inflammatory response and destroys bacteria. Therefore, MGO in manuka honey acts as an antiseptic to reduce bacterial load (Naik et al., 2021). In contrast, the non-peroxidase compounds (flavonoids and polyphenols) in stingless bee kelulut honey complemented with high acidity and high sugar content were reported to inhibit bacterial and fungus growth. Despite having different physiochemical compounds, all honey contains antibacterial properties to enhance wound healing outcomes (Omar et al., 2019). Besides having anti-bacterial properties, the immunomodulatory and antioxidant properties further extend the usage of honey in any part of the wound healing process (Angioi et al., 2021; Kus & Ruiz, 2020; Naik et al., 2022). Nevertheless, the broad-spectrum antibacterial activities with high susceptibility for *Staphylococcus aureus* displayed by stingless bee kelulut honey irrespective of geographical locality are potentially useful to overcome challenges in the management of antibiotic-resistant bugs (Ramón-Sierra et al., 2020).

However, it is worth noting that not all honey is the same and the potential benefits of honey are highly dependent on its source. Overall, honey has a major role to play in the treatment of non-healing DFU across healthcare settings (Alvarez-Suarez et al., 2018). The next section presents a literature review concerning the efficacy of honey for DFU treatment.

2.7.4 Honey in Malaysia

There are two main types of honey in Malaysia, namely monofloral honey (e.g., acacia, gelam, and pineapple) and poly floral honey (e.g., kelulut and tualang). Laboratory investigations indicate that all Malaysian honey has antibacterial potency that is similar to New Zealand's manuka honey (Ibrahim et al., 2016; Izzati Shahira et al., 2021; Ranneh et al., 2018). In the local context, kelulut honey from stingless bees and Tualang honey from honey bees are the most popular and generally consumed for good health (Ismail & Ismail, 2018; Mohamad et al., 2019). It was reported that Tualang honey has the closest resemblance to manuka honey. In recent years, there has been a resurgence of interest in Tualang honey due to its potential health benefits. Zainol et al. (2013) reiterated that studies on Tualang honey ranged from laboratory tests and animal experiments. However, human-related clinical studies with Tualang honey remain nil. According to a comparison study by Ranneh et al. (2018), manuka honey and Tualang honey originated from the same genus but differ based on species and floral origin. Tualang honey is a poly floral honey produced by the sting rock bees *Apis dorsata* which are incredibly aggressive bees. It is mainly found in huge combs hanging in open sp and situated upon the branches of very tall trees, *Koompassia excelsa (Becc.)*. This imposes a significant challenge to search and obtain Tualang honey (Ahmed & Othman, 2013).

Several studies demonstrated the therapeutic properties of Tualang honey against various microorganisms, including *Pseudomonas spp*. and MRSA (Molan, 2006; Pieper, 2009) but not on actual chronic wounds. Khalil et al. (2011) tested the antibacterial properties of Tualang honey against 13 different bacterial species in comparison to manuka honey that showed similarity in their antimicrobial activity against *Stenotrophomonas maltophilia* which was reported to be resistant to many broad-spectrum antibiotics such as carbapenems. However, Tualang honey was shown to be more effective than manuka honey

in inhibiting the growth of *Acinetobacter spp.* This pathogen was reported to have a high resistant profile and cause nosocomial infections, pneumonia, bacteremia, and even death (Piperaki et al., 2019). Overall, Tualang honey revealed a substantial antibacterial effect against Gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas spp*., and *Acinetobacter spp)* as compared to Gram-positive bacteria (*Staphylococcus aureus*, coagulase-negative, and *Streptococcus spp*). Hence, the outcome gave an indication regarding the potential efficacy of Tualang honey in the management of infected wounds. However, the difficulty in accessibility may have been a reason for this honey not being researched for clinical usage (Alam et al., 2014; Khalil et al., 2011; Nasir et al., 2010; Ngaini et al., 2018; Zainol et al., 2013). Furthermore, local clinical studies on the efficacy of Tualang honey in DFU may be lagging since it is categorised in the same group of species as manuka honey.

In recent times, honey produced by stingless bees has gained prominent research interest. Historically, stingless bees originated from Africa before they migrated to Europe, America, and Asia. Generally, stingless bees are native to tropical and sub-tropical areas in Southeast Asia including Malaysia (Selvaraju et al., 2019). Stingless bee belongs to the order Hymenoptera under the family Apidae and the sub-family tribe Meliponini and the most common stingless bees that are in the mainstream of research are from the *Trigona sp* and *Mellipona sp*. Furthermore, stingless bees have been identified as small non-aggressive bees from the species of *Trigona* or *Meliponiae.* The species is identified with different names in different regions (Maringgal et al., 2019). For example, it is known as "*damar*" in India and "*lukut*" in the Philippines. In Malaysia, the local stingless bees are called kelulut bees and 35 out of 500 species of stingless bees in the world can be found in Malaysia (Kek et al., 2017). Additionally, Quezada-Euán (2018) mentioned that stingless bee honey and its related products, such as propolis, wax, and pollen, are collected by communities across the world for medical remedies as well as cultural and religious activities.

According to Ismail and Ismail (2018), local kelulut beekeeping started in 2004, initiated by the Malaysian Agriculture Research and Development Institute (MARDI).

Majority of the stingless bees originated from sub-tropical regions. However, only four local kelulut bee species were successfully identified and included for beekeeping in the local context, namely *Trigona itama*, *Trigona apicalis*, *Trigona carnifrons*, and *Trigona thoracica* (Soh et al., 2021). The size of the bees ranges from 2 to 5 mm and they have no stingers. Previous studies denoted that stingless bees are highly sociable with one queen living together with thousands of workers (Kek et al., 2018; Zuccato et al., 2017). Figure 5 illustrates the stingless bees *Trigona itama* while its cerumen/honey pot is shown in Figure 6.

Figure 5: Stingless bee *Trigona itama*

Figure 6: Cerumen/honeypot of stingless bee *Trigona itama*

Several publications have demonstrated sizeable evidence concerning the medicinal and therapeutic effects of stingless bee honey across different stingless bee species (Aina et al., 2018; Carina Biluca et al., 2021). Honey produced by multiple species of stingless bees has shown great potential for consumption and commercialisation due to its higher phenolic, antioxidant, and flavonoid content as compared to honey bees *Apis mellifera*. Abdul Karim and Anum (2019) stated that kelulut honey was low in quantity back in the 20th century and thus, honey bees were more popular as the main producer of honey in many countries including Malaysia. Conversely, the availability of kelulut honey was not as widespread as honeybees and exploration of its therapeutic properties took a back step. As kelulut honey began to penetrate the market for nutritional purposes, research on the physiochemical and phytochemical properties began. The findings in previous evaluations showed kelulut honey to be slightly different from common honey bees in terms of composition and phytochemical attributes mainly in terms of taste, viscosity, sugar content, water content, and color (Chong et al., 2021; Esa et al., 2022; Mohamad et al., 2019).

Given the similarities in the therapeutic properties with the honey bees of *Apis spp*, kelulut honey may be useful in improving wound healing outcomes (Rao et al., 2016).

However, research on kelulut honey in wound healing has been undeniably lacking despite showing greater anti-oxidant properties as compared to manuka honey. Thus, the potential of kelulut honey in the local context has been under-explored (Imtiazah et al., 2021; Selvaraju et al., 2019). Despite the increase in incidences of foot complications due to non-healing wounds DFUs, exploration into honey for wound management remains sparse in Malaysia (Abdul Karim & Anum, 2019). Nevertheless, research on the physiochemical of kelulut honey has been forthcoming due to the surge in the kelulut beekeeping activities. In a recent study by Rosli et al. (2020), locally produced kelulut honey from stingless bees *Trigona itama* showed superior antimicrobial potency due to high levels of antioxidants against five bacteria; and most importantly *Staphylococcus aureus* which is quite predominant in non-healing-DFU. Despite not gaining momentum in the clinical field, the kelulut industry has recently gained wide attention as it was listed as a Malaysian superfood by MARDI in 2016 (Ismail & Ismail, 2018). In the 21st century, beekeepers of kelulut honey in Malaysia resurged since the handling of these bees was found to be more user-friendly as compared to honey bees that sting. It was also revealed that kelulut bees in Malaysia produced more honey than honey bees *Apis spp* from Australia and New Zealand (Yaacob et al., 2017). Consequently, exploration into the rearing of kelulut bees as an economic proposition has increased the production of kelulut honey in the local market. As the quantity of kelulut honey increased, research into its nutritional values gained momentum. Recent findings showed promising outcomes demonstrating similar nutritional value between kelulut honey and manuka honey. Hence, kelulut honey was introduced as a superfood for maintaining good health (Mustafa et al., 2018; Rozman et al., 2022).

The exploration of kelulut honey has led to studies confirming its efficacy in disease management and inhibition of bacterial growth, with an additional effect on angiogenesis and oxygen circulation (Abd Jalil et al., 2017). Moreover, recent studies on the physiochemical properties of kelulut honey indicated that the minimum inhibition concentration (MIC) value (an indicator for the antibacterial properties of honey) was the lowest in kelulut honey compared to manuka honey, propolis, and artificial honey. Therefore, clinical applications of kelulut honey may find a place in healthcare settings to combat the ever-growing antibiotic resistance issues in the treatment of non-healing DFU.

Few studies have demonstrated the physiochemical properties of kelulut honey consisting of higher anti-oxidant properties compared to *Apis spp*. and this finding strengthened the potential utilisation of kelulut honey for the treatment of chronic wounds, especially DFU (Omar et al., 2019; Ulasan et al., 2020). There have been positive revelations regarding kelulut honey despite the recognition received by medical-grade manuka honey in the treatment of wounds. In general, the efficacy of kelulut honey is based on the total high phenolic content which has been proven to produce promising anti-oxidant, anti-inflammatory, and antimicrobial effects. The therapeutic benefits of kelulut honey could reduce oxidative stress, increase free radical scavenging activity, control inflammation, reduce edema, bactericidal to stimulate bactericidal protein production, DNA replication and cellular metabolism, and bacteriostatic to inhibit cell wall synthesis (Abdul Karim & Anum, 2019; Maringgal et al., 2019; Ranneh et al., 2018).

Anti-oxidant activity has been considered one of the major components of wound healing to reduce oxidative stress, and prevent cell damage at the wound site. In terms of therapeutic properties, the anti-oxidant capacity of kelulut honey was proven far superior to honey bees. Higher antioxidant properties for *Trigona sp* as compared to *Apis mellifera* had produced excellent outcomes in reducing inflammation and controlling infection (Alvarez-Suarez et al., 2018; Gill et al., 2019; Ibrahim et al., 2016). In similar studies, kelulut honey demonstrated a higher concentration of anti-oxidant compounds (flavonoids) than honey bees. In almost all past literature on stingless bee honey, the phenolic acid and flavonoids of kelulut honey were shown higher than *Apis spp* (Abu Bakar et al., 2017; Kek et al., 2014). Study by Ranneh et al. (2018) also concurred with earlier findings where kelulut honey from *Trigona itama* showed higher anti-oxidant capacity using liquid chromatography-mass spectrometry compared to local Tualang honey, *Apis dorsata*. About 18 phenolic acids and flavonoids were isolated from kelulut honey and were found to have higher vitamin C, protein, and polyphenols concentrations compared to Tualang honey. Given the promising findings, kelulut honey can be recommended for therapeutic usage in wound healing as incidences of foot complications and antibiotic resistance are on the rise (Aina et al., 2018; Boorn et al., 2010; Kek et al., 2014; Oddo et al., 2008; Rashid et al., 2019). In addition, research conducted by MARDI indicated the major free phenolic acid

in the kelulut honey comprised of protocatechuic acid and 4-hydroxyphenylacetic acid that can act as a strong anti-oxidant to enhance cell proliferation and fit for wound healing (Ismail & Ismail, 2018).

Amidst the promising outcomes of kelulut honey, it is worth noting that total phenolic content differs among the honey regarding the type of bee species, region, season, and type of floral sources. One of the first comparative studies on three types of honey in Malaysia evaluated the phytochemical (total phenolic acid and flavonoids) and anti-oxidant properties was conducted by Maringgal et al. (2019). It was an interesting study where all three kelulut honey were sourced from five different parts of Malaysia and the anti-oxidant compound was evaluated based on the 1,1-diphenyl2-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing antioxidant power assay (FRAP). The result showed there were significant differences in phytochemical compositions and antioxidant activities despite the kelulut honey being produced in the same country.

The finding concurred with a previous study by Nordin et al. (2018) where it was highlighted that the phytochemical of common honey could vary depending on the geographical location in Malaysia. However, the outcome of the study squashed the assumption that honey from the same region may produce similar antioxidant properties (Biluca et al., 2016; Braghini et al., 2021; Carina Biluca et al., 2021; Silva dos Santos et al., 2021). Thus, it can be concluded that factors such as geographical location and cultivation process may affect the phytochemical composition and antioxidant capacity of kelulut honey. Nevertheless, kelulut honey has been consistently shown to possess high phytochemicals (phenolic acid and flavonoids) which contributes to stronger anti-oxidant capacity. Furthermore, phenolic compounds such as caffeic and ferulic acids formed the base for the anti-oxidant capacity of kelulut honey which had been highlighted in previous studies (Al-Hatamleh et al., 2020). Therefore, the phenolic compounds have been much studied since it is the science behind the anti-oxidant, antibacterial, and anti-inflammatory properties of kelulut honey that may positively reduce inflammation and inhibit macrophage inflammatory protein-2 (MIP-2). A study by Moniruzzaman et al. (2014) proved that phenolic acid from stingless bee honey could reduce the production of reactive

oxygen species (ROS) by 55 ± 14% and produce anti-inflammatory activity. Despite being an in vivo study, it was the landmark study indicating the efficacy of stingless bee honey as an anti-inflammatory agent. Despite the positive findings on the anti-inflammatory effect of kelulut honey, the evaluation approaches were mostly animal-based in vivo studies (Ibrahim et al., 2016).

2.8 LITERATURE REVIEW

In this section, evidence-based resources were sought to give an updated review of published articles on honey in the treatment of DFU.

2.8.1 Framework for Literature Search

The PICO framework (Patient(P), Intervention(I), Comparison(C), and Outcome(O)) was used as the foundation for the literature search in this study. This framework efficiently identifies the required information to formulate relevant questions to set the focus for the literature search (Eriksen & Frandsen, 2018). For this research, P (patient) described an adult patient above 18 years of age with DFU, I (intervention) referred to the topical application of honey on DFU, Comparison (C) referred to other topical dressings, and O (outcome) referred to the healing efficacy of honey and associated factors influencing the healing efficacy of honey such as the patients' socio-demography, clinical characteristics, and wound characteristics. The PICO framework of the literature search is described in Table 3.

Table 3: PICO Framework for Literature Review

Patient (P)	**I**ntervention **(I)**	**C**omparison **(C)**	**O**utcome **(O)**
Adult patients more than 18 years old with DFU.	Topical application of honey on DFU.	DFU treated with other topical dressing or conventional dressing.	Healing outcome Association of patient socio-demography, clinical and wound characteristics with the healing efficacy.

2.8.2 Research Questions for Literature Review

Based on the PICO framework, relevant questions were formulated to search for eligible studies. The research questions for the literature review are described below:

1. What are the characteristics of the patient population with DFU?
2. What are the healing outcomes anticipated with the topical application of honey on DFU?
3. What is the healing outcome anticipated in comparison studies between honey and other topical dressing or conventional dressing in DFU?
4. How do the patients' socio-demography, clinical, and wound characteristics affect the healing outcome in DFU with the intervention of honey?

2.8.3 Literature Search Strategy

The related articles were searched through electronic databases via PubMed, Science Direct, Google Scholar, EBSCO, CINAHL, and Cochrane Library as these databases provided a more in-depth, significant, and comprehensive source for the literature search. As the present study was aimed at assessing the efficacy of stingless bee honey in DFU,

relevant terms such as wound healing, healing outcome, healing trajectory, DFU, kelulut, manuka, honey dressing, conventional dressing, topical agents, honey-impregnated gauze dressing, advanced dressing, antimicrobial dressings povidone dressing, saline dressing, patient's socio-demographic factors, and risk factors for wound healing were included in the literature search. The inclusion and exclusion criteria were included to further refine and strategise the literature search. The scope of the literature search was limited to publications in English as the translation of other languages to English may require a longer time. As studies pertaining to stingless bee honey or kelulut honey particularly in wound healing were limited, studies related to the efficacy of honey in the treatment of DFU published between 2012 to 2023 were included in the literature review. For the inclusion criteria, only human studies involving diabetic patients above 18 years of age with DFU were included in the search. RCT, experimental, prospective studies, and topical application of honey as an intervention were included in the search for eligible literature. The inclusion criteria for the literature search are summarised in Table 4.

Table 4: Inclusion Criteria for Literature Search

Inclusion criteria
Human studies
Adult diabetic population more than 18 years old
Diabetes-based foot ulcerations (DFU)
Randomised controlled trials (RCTs), prospective interventional studies, quasi-experimental studies
Topical honey-based wound dressing
Comparative studies

2.8.4 Findings of Literature Search

The search for literature related to the efficacy of honey in wound healing which corresponded to the inclusion and exclusion criteria were selected and the full texts were reviewed. A total of 153 articles related to the efficacy of honey were identified using electronic databases. However, 90 articles consisting of duplicated articles and animal studies were excluded from the search. The abstracts and titles of the remaining 63 articles were screened and checked for eligibility and 39 unrelated articles were removed based on non-targeted population, outcomes, laboratory-based studies, bulletins, and newsletters. Full-text reading was conducted on the shortlisted 24 studies, resulting in the exclusion of 14 studies with bee products like propolis and royal jelly. Finally, 10 articles that were consistent with the eligibility criteria were reviewed.

Figure 5 illustrates the selection process of articles based on the PRISMA Flow Diagram (Page Mathew et al, 2021) (Preferred Reporting Items for Systematic Reviews & Meta-Analyses).

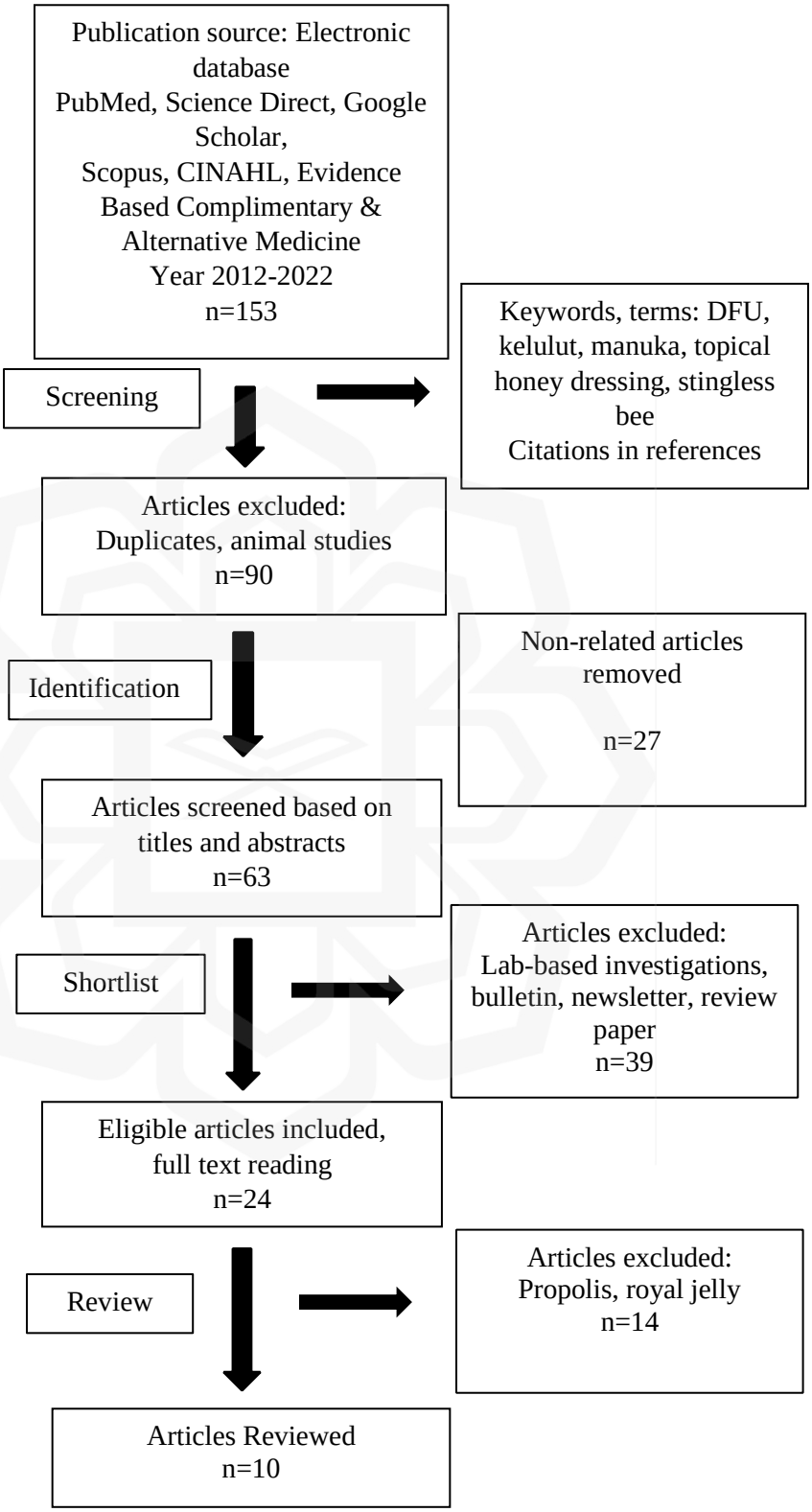

Figure 5: The eligible articles selection process based on PRISMA

2.8.5 Characteristics of the Eligible Articles

There was a lack of studies related to the efficacy of kelulut honey in wound healing. Comparative studies between honey and conventional methods or other treatments were also limited. There were no comparative studies between different types of honey in the healing of DFU. Overall, only a handful of comparative studies were undertaken in several countries such as India, Pakistan, Iran, Indonesia, Greece, China, and Saudi Arabia for the past ten years. In the local context, no study was conducted in Malaysia at the point of investigation. Therefore, knowledge on the efficacy of honey-based wound dressing, particularly stingless bee honey, in the treatment of DFU remains elusive. A summary of the reviewed studies is shown in Table 5.

Table 5: Summary of Reviewed Articles

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Reference	Year	Country	Type of honey	Comparator	Sample	Study Design
Jan et al.	2012	Pakistan	Unspecified	Pyodine dressing	100	Quasi-experimental study
Kamarotos et al.	2014	Greece	Manuka honey (impregnated dressing)	Saline dressing	63	RCT
Imran et al.	2015	Pakistan	Sterile undiluted Beri honey (impregnated dressing)	Saline dressing	348	RCT
Agarwal et al.	2015	India	Pure (undiluted)	Povidone dressing	36	RCT
Tsang et al.	2017	China	Manuka honey	Nanocrystalline silver (NS); paraffin tulle	31	RCT
Bashir et al.	2018	Pakistan	Unspecified	negative pressure wound treatment	93	RCT
Al Saeed et al.	2019	Saudi Arabia	Manuka honey	Silver dressing	71	RCT
Koujalagi et al.	2020	India	Unprocessed	Povidone dressing	64	RCT
Karimi et al.	2019	Iran	Unspecified	Conventional treatment olive oil	45	RCT
Chauhan et al.	2020	India	Unspecified	Betadine dressing	60	Prospective-controlled study

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2.9 CRITICAL APPRAISAL OF LITERATURE

Several appraisal tools have been designed to critically appraise the reliability, validity, and relevance of clinical evidence. For the present study, 10 quantitative studies were appraised using the Joanna Briggs Institute appraisal tools that were deemed appropriate for the research design (Zeng et al., 2015). Adhering to the JBI appraisal standards, the quality of the studies was assessed using the JBI critical appraisal checklist and classified as high quality with a score of 70% and above whereas studies with scores between 50% to 69% and lower than 50% are of medium and low quality (Barker et al., 2023). The scores were adjusted based on 10 questions deemed related to RCT and 9 questions for non-randomised controlled trials. Based on the JBI appraisal scoring, there were six high quality studies (Al Saeed, 2019; Jan et al., 2012; Kamaratos et al., 2014; Karimi et al., 2019; Koujalagi et al., 2020; Tsang et al., 2017) and the remaining four were categorised as medium quality studies (Agarwal et al., 2015; Bashir et al., 2018; Chauhan et al., 2020; Imran et al., 2015). The explanation for the JBI critical appraisal tool for RCTs is attached in Appendix A1 and for the non-randomised controlled trials in Appendix A2. The critically appraised studies based on JBI appraisal tools are summarised in Table 6 for RCT and Table 7 for non-randomised controlled trials.

Table 6: **JBI Critical Appraisal Tool For RCT**

Author	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Quality of the study(%)
Kamaratos et al. (2014b)	UC	X	✓	✓	✓	✓	✓	X	✓	✓	70
Imran et al. (2015a)	✓	✓	✓	X	X	UC	✓	X	✓	✓	60
Agarwal et al. (2015)	✓	UC	✓	UC	UC	✓	X	UC	✓	✓	50
Tsang et al. (2017b)	✓	✓	✓	X	X	✓	✓	✓	✓	✓	80
Bashir et al. (2018)	✓	✓	✓	X	X	UC	X	✓	✓	X	50
Al Saeed (2019)	✓	✓	✓	✓	✓	UC	✓	✓	✓	✓	90
Karimi et al. (2019)	✓	✓	✓	X	X	UC	✓	✓	✓	✓	70
Koujalagi et al. (2020b)	✓	✓	✓	X	X	X	✓	✓	✓	✓	70

✓:Yes X:No UC:Unclear

Q1: Was true randomisation used for the assignment of participants to treatment groups?

Q2: Was allocation to treatment groups concealed?

Q3: Were treatment groups similar at the baseline?

Q4: Were participants blind to treatment assignment?

Q5: Were those delivering treatment blind to treatment assignment?

Q6: Were outcomes assessors blind to treatment assignment?

Q7: Were treatment groups treated identically other than the intervention of interest?

Q8: Were outcomes measured in a reliable way?

Q9: Was follow-up complete and if not, were differences between groups in terms of their follow-up adequately described and analysed?

Q10: Was appropriate statistical analysis used?

Table 7: **JBI Critical Appraisal Tool For Non-Randomised Controlled Trials**

Author	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Quality of the study (%)
Jan et al. (2012)	✓	✓	✓	X	✓	✓	✓	✓	UC	78
Chauhan et al. (2020)	✓	✓	✓	X	✓	UC	✓	UC	✓	67

✓: Yes X: No UC: Unclear

Q1: Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?
Q2: Were the participants included in any comparisons similar?
Q3: Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?
Q4: Was there a control group?
Q5: Were there multiple measurements of the outcome both pre and post the intervention/exposure?
Q6: Was follow-up complete and if not, were differences between groups in terms of their follow-up adequately described and analysed?
Q7: Were the outcomes of participants included in any comparisons measured in the same way?
Q8: Were outcomes measured in a reliable way?
Q9: Was appropriate statistical analysis used?

2.10 RESULT

Based on the reviewed studies, there were eight RCTs, one quasi-experimental study, and one prospective controlled study. As shown in Table 6, the studies were either performed with unsterilised, manuka honey or natural, native honey in different geographical settings across the globe. On average, the sample size ranged from 27 to 348 adult diabetics above 18 years of age. Majority of the studies compared the efficacy of honey to conventional dressings containing povidone-iodine, gauze-soaked saline dressing, olive oil, and advanced dressing with nanocrystalline silver and negative-pressure vacuum therapy. Time to healing based on wound size reduction, healing rate, disinfection rate, granulation, and epithelialisation rate were the main outcomes observed in these studies. However, comparative studies between different types of honey in the treatment of DFU remain sparse.

2.10.1 Assessment of Eligible Studies

Based on the reviewed studies, all 10 quantitative studies demonstrated clear aims and objectives supplemented with in-depth background knowledge related to DFU and the efficacy of honey in the treatment of DFU. The abstract and introduction gave an overall picture of the approach of the researchers in their respective studies. Overall, honey was shown to be effective in the healing of DFU as compared to conventional treatment as shown in the eligible studies except for two studies that showed silver dressing and negative-pressure vacuum therapy superior to honey (Bashir et al., 2018; Tsang et al., 2017). Nevertheless, there were several limitations identified in the methodologies of the reviewed studies. `

2.10.2 Efficacy of Honey vs. Conventional Treatment

Jan et al. (2012) evaluated the efficacy of honey compared to pyodine dressing in a quasi-experimental study with a total study population of 100 diabetics with Wagner

classification I-IV. A significant higher healing rate was observed with the intervention of honey as compared to conventional pyodine dressing (72% vs. 66%) in the observation period of a year. The amputation rate was revealed to be lower in the honey group as compared to the conventional group (28% vs. 34%). Overall, the healing outcome was significantly faster in DFUs treated with honey dressing. Hence, it was concluded that honey was more effective than conventional dressing using pyodine dressing in DFU. Despite the significant findings, there was no information on the type of honey used in the study and how the honey was applied to the wounds. It is crucial to define the type of honey used in the study to ascertain the therapeutic properties as not all honey is similar (Muñoz et al., 2023).

Building on the findings, RCT was undertaken by Kamaratos et al. (2014) to compare the efficacy of manuka honey-impregnated dressing and conventional dressing using normal saline among 63 participants with neuropathic DFUs for 16 weeks. The outcome of the study was consistent with Jan et al. (2012) whereby the time to healing was shorter in the intervention honey group as compared to the conventional group (31 ± 4 days vs 43 ± 3 days) but the outcome was not statistically significant. The finding also indicated manuka honey-impregnated dressing as a potent disinfectant as none of the DFUs treated with manuka honey required antibiotics during the study. This randomised, double-blinded study with a control group has certainly strengthened the antibacterial efficacy of manuka honey dressing in the treatment of DFU. However, the randomisation process in the study lacks information and may contribute to bias. Despite the study mentioning that the participants were randomly assigned, there was no method used to assign the participants to intervention and control groups as the first participant was assigned to Group 1 and another to Group 2 alternatively.

A similar RCT with one of the highest number of participants (n=348) was performed by Imran et al. (2015) with Beri honey. The study showed healing time was shorter in DFU treated with Beri honey (from *Apis sp*) as compared to conventional saline dressing (18 days vs. 29 days). Furthermore, 75.97% of the DFUs treated with honey achieved complete healing as compared to 57.39% in the conventional group. Despite

showing similar healing outcomes with previous studies by Jan et al. (2012) and Kamaratos et al. (2014), the study by Imran et al. (2015) had a bigger sample size (n=348) and was conducted in 2 hospitals, thus adding higher reliability for its findings. However, the study population was only pooled from a lower-income stratum, making the outcome non-generalisable across the various segmentation of society. In addition, no blinding was performed during the study, which is highly required in RCT. On another note, it was mentioned in the methodology that undiluted honey was used which may have affected the amount of honey on the wound bed as it could have stuck on the gauze applied as a primary dressing. As no detailed information was given in the study protocol, the procedure for honey application was unclear. The point to be stressed here is this RCT has the biggest sample size and the longest study duration of 4 years in a honey-related study for the treatment of DFU. Given the sample size and comprehensive study protocol, the differences in the healing time between both the honey and conventional groups were statistically significant ($p<0.001$). In comparison, the duration of healing is shorter than Kamaratos et al. (2014). Despite methodological limitations, the study by Imran et al. (2015) highlighted the importance of using sterilised honey for wound healing to eliminate contaminants and bacteria such as *Clostridium sp* spores which may cause weakness of muscle and paralysis.

The potential of honey as wound dressing is further strengthened by another RCT by Agarwal et al. (2015) with a shorter healing time demonstrated by pure undiluted honey as compared to povidone-iodine dressing (14.2 days vs. 15.5 days). However, the finding was not statistically significant. Secondary outcomes in this study included reduction in edema, pain, and exudate with the honey dressing. More positive outcomes were observed with honey dressing where participants endured less pain during dressing change in the honey group as honey maintains moisture in adherence to the wound bed. Furthermore, the participants were followed up for 120 days, which is the targeted timeline to observe the healing trajectory of DFUs as demonstrated in other DFU-related studies (Everett & Mathioudakis, 2018; Guest et al., 2018; Ugwu et al., 2019). Thus, it was concluded that honey was a potentially safer wound dressing compared to povidone dressing. In contrast to Imran et al. (2015), single blinding was included in the study protocol of Agarwal et al. (2015) to minimise the factor of bias. Nevertheless, there was a similarity between Agarwal

et al. (2015) and Imran et al. (2015) based on the undiluted honey that was used in both studies, which may have affected the standardisation of the amount of honey applied to the ulcer. The sample size was also smaller (n=36) and was conducted in a single centre, which could be argued on the generalisation of its findings.

A recent multi-centered RCT by Koujalagi et al. (2020) showed similar findings as Agarwal et al. (2015) whereby honey was more effective than conventional povidone dressing in reducing wound size in 15 days. The mean wound size for the honey group was significantly reduced on day 15 from baseline (23.16 cm^2 to 10.69 cm^2) compared to conventional povidone dressing (23.03 cm^2 to 15.06 cm^2). There was no statistical difference in wound size reduction on days 3, 5, 7, and 10 from baseline between both groups. However, the size reduction was statistically significant only on day 15. In this study, the research population included farmers, workers, and bus drivers. However, the rationalisation for specifically choosing these groups for the RCT was not elaborated. There were 18 farmers, 10 workers, and 4 bus drivers in the honey group whereas there were 15 farmers, 14 workers, and 3 bus drivers in the conventional group. While the occupational categorisations did not differ statistically between both groups, other factors such as the nature of the job, hygiene, and exposure to different kinds of environment may have affected the results. Blinding was also not mentioned in the study despite it being an RCT. Furthermore, there was no homogeneity in the gender factor of the research population as there were more males (n=50) than females (n=14). However, the relationship between confounding factors, such as gender, types of occupations, and healing outcomes, was not assessed in the study. The outcome may have been influenced by these factors, which were not explored. The outcome of the study revealed the potential of honey in stimulating granulation and epithelialisation for faster healing in DFU as compared to conventional povidone dressing that could hamper the healing process due to its corrosiveness. However, the findings may lack reliability as a transparent graph paper was used to measure the size of the ulcer in the study which may lack reliability and validity. Hence, the findings in the previous study could have suffered due to the unavailability of more accurate instruments for wound measurement in healthcare settings. Similar limitation was observed where a ruler was used by Kamaratos et al. (2014) and Imran et al. (2015), which could have caused

human error during measurement and a lack of consistency when different staff handled the measurement procedure.

In contrast to the previous studies mentioned above, a study by Karimi et al. (2019) compared the efficacy of honey (heated) to conventional olive oil dressing. The potential of olive oil in wound healing was reported to be attributed to the phenolic compounds in olive oil, which are like the properties of honey (Taheri & Amiri-Farahani, 2021; Visioli et al., 2020). The healing outcome was measured based on the wound healing score. Using this score, it was concluded that the effect of heated honey was comparable to olive oil. Since both honey and olive oil have similar therapeutic properties, the outcome was anticipated with a score of 267.5 (before) to 371.5 (after 1 month) in the honey group as well as 253.0 and 330.5 in the olive oil group. This could be attributed to the small sample size of less than 30 patients in each group. In this study, there were several absences of information on randomisation and blinding process which was not included in the study. Another factor that could have influenced wound healing was heating the honey before application as studies had shown heating could destroy the antioxidants and enzymes the nutrients in the honey (Annapoorani et al., 2010). Therefore, the lack of methodological information hampered the effort to evaluate the study objectively.

The efficacy of honey was further investigated by Chauhan et al. (2020) and the primary outcome included time to healing based on the appearance of granulation and epithelialisation. Similar to previous studies, honey was more effective than betadine dressing (Agarwal et al., 2015; Imran et al., 2015; Jan et al., 2012; Koujalagi et al., 2020). The time to granulation was 4 days in the honey group and 7 days in the betadine group whereas the time to epithelialisation was 9 days as opposed to 12 days in the betadine group. Despite the positive outcomes, there was a lack of details in the study design related to tools used to measure the outcomes in the study. Furthermore, details on the personnel involved in the data collection were not mentioned. Also, there was a lack of information in the publication on the randomisation and blinding process.

Overall, the mentioned studies showed that the effectiveness of honey was superior to conventional dressings either with saline dressing or povidone dressing (Agarwal et al.,

2015; Chauhan et al., 2020; Imran et al., 2015; Jan et al., 2012; Kamaratos et al., 2014; Koujalagi et al., 2020) and one comparable study with olive oil (Karimi et al., 2019). Hence, the findings from these previous studies indicated that honey is potentially effective in improving healing outcomes in the treatment of DFU as opposed to conventional treatment due to its natural anti-bacterial, anti-inflammatory, and antioxidant properties (Wang et al., 2019).

2.10.3 Efficacy of Honey vs. Advanced Dressing

Advances in wound care have brought newer dressings to the fraternity of wound management in DFU to produce a better healing outcome as compared to conventional dressings. Comparative studies between conventional studies and advanced dressing or therapy have been forthcoming in recent times (Rezvani Ghomi et al., 2019).

Expanding the knowledge from previous comparative studies, Bashir et al. (2018) evaluated the efficacy of honey compared to negative pressure vacuum therapy across a research population of 95 participants. Unlike previous studies, their findings showed that negative pressure vacuum therapy was more effective than honey in the treatment of DFU. The number of days taken to observe healthy granulation tissue in the honey group was slower (28.8 days) as compared to the group treated with negative pressure vacuum therapy (18 days). However, the differences in wound size for both groups at baseline were not similar, which could have affected the outcome as wounds in the group treated with negative pressure vacuum were much smaller (19 cm^2) than the honey group (23 cm^2). Furthermore, there were limitations in the information provided in the methodology on the randomisation process, blinding information, and negative pressure vacuum dressing changing protocol. The reliability of using a measuring tape to measure wound sizes could be questionable in terms of accuracy and consistency throughout the study. More accurate and approved tools could have been used for measurement purposes. While statistical analysis using SPSS version 20 was mentioned, the testing outcomes were not included which brought forward the question if the results were significant differences between the two groups despite the result showing faster granulation in the negative pressure vacuum

group. Based on the study protocol, patients either bought the portable negative pressure vacuum therapy and were taught how to handle it at home or patients stayed in the ward with in-house vacuum therapy for those who could not afford the machine. The standardisation of therapy was not observed as patients were observed in two different environments during the study. Hence, these factors could affect the reliability and contribute risk of bias to influence the outcome of the study. Therefore, a more comprehensive study protocol is required to confirm the findings as not much data is available on comparative studies between negative pressure vacuum therapy and honey in wound healing. Undoubtedly, both modalities were beneficial in improving the healing outcome of DFU but this study was one of the first to show that negative pressure vacuum therapy was more effective than honey in the healing of DFU.

In a pilot randomised 3-way-comparative study, Tsang et al. (2017) compared the efficacy of nanocrystalline Ag, manuka honey, and conventional paraffin tulle dressing in the healing of DFU. The signs of infection and bacteriology assessment via wound swabs were also assessed during the study period of 12 weeks. The result revealed wound size reduction rate was the highest in the nanocrystalline silver Ag group (n=11, 97.45%) followed by manuka honey (n=10, 86.24%), and conventional dressing using paraffin tulle (n=10, 76.91%) in 12 weeks. The findings were similar to Bashir et al. (2018) where honey was less effective than the comparator. Tsang et al. (2017) reported that the efficacy of advanced dressing using nanocrystalline silver Ag was superior to manuka honey in the treatment of DFU. However, manuka honey was shown to be more effective than conventional dressing using paraffin tulle in the same study. The outcome on the cumulative healing incidence was 81.8% in nanocrystalline silver Ag, 50% in the manuka honey group, and 40% in the conventional group. Clinical signs of infection and bacteriology were not significantly different among the three groups despite the marginal mean microorganism being different among the three groups with microorganisms in the conventional group being the highest (1.36), followed by manuka honey (1.17) and lowest among the nanocrystalline Ag group (0.86). Thus, it was concluded the antibacterial clearance of nanocrystalline Ag was superior to manuka honey and manuka honey was more effective than conventional dressing as the load of bacteria was the most reduced in

the wounds treated with nanocrystalline silver Ag and least reduced in conventional dressing in this study. However, the cost-effectiveness of using nanocrystalline Ag was a matter of concern as nanocrystalline silver dressing is 1.3 times higher than the conventional dressing. Similarly, the cost of manuka honey was 1.1 times higher than the conventional dressing. Hence, the affordability of patients may be a factor of concern when these materials were chosen for wound treatment as compared to conventional dressing. Thus, conventional dressing was preferred in healthcare settings to reduce the burden of healthcare as well. Manuka honey dressing also could be considered as it is cheaper compared to nano-crystallised silver dressing. The limitations were in the study protocol which lack of information provided on the amount of nanocrystalline Ag, honey, and paraffin tulle applied to the DFUs during the study. The small sample size of less than 30 and the different stages of DFU grade among the three groups may have contributed to the factor of bias and non-significant outcomes in the study. Nevertheless, comparative studies between nanocrystalline Ag and. honey amounts to further evaluations on a larger scale.

In a similar study, Al Saeed (2019) compared the efficacy of manuka honey-impregnated dressing (35g) with silver hydrogel dressing (controlled-release) among diabetic participants with neuropathic DFUs within 2 years. The outcome was in contrast with (K. K. Tsang et al., 2017) where manuka honey was found to be more effective than silver-hydrogel dressing in terms of the mean duration taken to achieve complete healing of DFU. However, silver hydrogel dressing was more effective than manuka honey dressing in terms of infection rate reduction and duration of hospitalisation. Despite the differences, the finding was not statistically significant between both groups. The differences in the findings by Tsang et al. (2017) and Al Saeed (2019) could be due to the chemical compositions even though both have similar mechanisms of action in removing bioburden on the wound bed. Different types of silver-based dressing preparations may induce different therapeutic responses in the eradication of bioburden on infected wounds. Nano-crystalline silver is presumably smaller with higher solubility as compared to ionic silver gel. As limited studies have investigated the preparation of silver for wound healing purposes, more investigations are warranted (Gunasekaran et al., 2012). Therefore, the type of silver matters in concluding the efficacy of silver in wound healing and more evaluations

are needed to verify the findings. Nevertheless, the utilisation of silver showed greater anti-microbial potential, reduced the number of daily dressing changes, and was less toxic which is arguable as a lack of evidence (Dumville et al., 2017). Given the location of the DFUs on the plantar aspect, the findings by Al Saeed (2019) may not be generalised as offloading information in the study protocol was not elaborated, which is an important factor that could influence the healing trajectory of DFU (Lim et al., 2017; Wang et al., 2022). Nevertheless, the outcomes by Al Saeed (2019) further strengthened the efficacy of manuka honey dressing in achieving positive healing outcomes in DFU, which is aligned with Kamaratos et al. (2014). It also verifies the effect of silver-based dressing in reducing infection rate among infected DFU. In comparison, the healing proportion was higher in the study by Al Saeed (2019) as compared to Tsang et al. (2017), which could be attributed to surgical procedures conducted to remove gangrene or infected toes among the participants during the study. Hence, the time to healing was expedited. Despite using manuka honey in both studies, the outcomes differed due to different types of silver dressing used as comparator. Therefore, the outcomes could not be generalised between the two studies.

Based on the articles, honey is more effective than conventional dressings in the treatment of DFU as mentioned earlier. As for the comparison between honey and advanced treatments like nano-crystallised silver dressing and negative pressure vacuum therapy, further investigations are required to confirm the findings. There is certainly a lack of comparative studies using different types of honey in the treatment of DFU. In the context of stingless bee honey, clinical studies related to wound healing remain sparse despite being proven to have similar therapeutic properties as honey bees *Apis mellifera* or manuka honey (Abd Jalil et al., 2017; Al-Hatamleh et al., 2020; Ávila et al., 2018; Mohamad et al., 2019; Pimentel et al., 2021; Ramón-Sierra et al., 2020; Rao et al., 2016; Souza et al., 2021). Considering the absence of clinical studies with stingless bee honey in the healing of wounds especially DFU, future investigations are needed to establish the healing trajectory of DFU with stingless bee honey against other types of honey-based dressing or other conventional dressings like povidone iodine, saline, or even advanced dressing (silver or negative pressure vacuum therapy).

2.10.4 Associated Factors Related to the Healing of DFU

Foot complications can be prevented if the risk factors for DFU are identified and managed efficiently (Alsabek & Abdul Aziz, 2022; Armstrong et al., 2017; Rosedi et al., 2022). Given the identification of risk factors, implementation of diabetes foot care programs can be initiated to improve the healing outcomes and social aspects of the patients' lives. Thus, the healthcare burden can be reduced (Ouyang et al., 2021). In the past two decades, the associated risk factors related to wound healing have been investigated in various studies to enhance the healing trajectory of DFU (Al-Mohaithef et al., 2022; Yazdanpanah et al., 2018). However, the associated factor related to wound healing with honey remains less common.

Based on the reviewed articles, only two studies explored the associated factors related to wound healing with honey in DFU. The secondary objective of Al Saeed (2019) assessed the patients' socio-demography, and clinical and wound characteristics associated with the healing of DFU. The outcome demonstrated a significant correlation between age and diabetes control (HbA1c reading) with the duration of wound healing in DFU. This outcome is consistent with majority of studies on DFU where age and HbA1c were highlighted as the independent risk factor for the healing trajectory of DFU. Older age above 65 years old and HbA1c more than 10 which indicated poor control of diabetes were shown as prognostic factors for lower limb amputations (Christman et al., 2011; Huang et al., 2019; Lin et al., 2020). However, the duration of diabetes and gender were not associated with healing time in the study, which has been reported inconsistently in several DFU-related studies (Al-Rubeaan et al., 2015; Moura Neto et al., 2013; Smith-Strøm et al., 2017). The presence of granulation tissue, the condition of wound edge and discharge were reported as independent risk factors for the healing of DFU by Koujalagi et al. (2020). However, the outcomes based on these factors were not elaborated in the result section. Therefore, no conclusion could be made on the association of the presence of granulation tissue, condition of wound edge, and discharge with the healing outcome of DFU. Other studies in the literature review did not investigate the associated factors related to wound healing with honey in DFU.

2.10.5 Methodological Limitations of the Reviewed Articles

Ten eligible articles were reviewed and several methodological limitations were identified, despite the elaborations on the aim, background, and methodologies. One of the major limitations in the reviewed studies was the study protocol. Firstly, the type of honey was not well defined in the study protocol. While few used manuka-honey-impregnated dressings (Al Saeed, 2019; Kamaratos et al., 2014; Tsang et al., 2017), others tended to use natural, undiluted honey (Agarwal et al., 2015; Bashir et al., 2018; Jan et al., 2012; Karimi et al., 2019), unprocessed honey (Koujalagi et al., 2020), natural sterile Beri honey (Imran et al., 2015). Whereas, two studies (Bashir et al., 2018; Chauhan et al., 2020) did not specify the type of honey used. Hence, the type of honey used across the reviewed articles was not uniform to provide consistent findings. Thus, the type of honey should be defined as different honey may produce different therapeutic outcomes (Almasaudi, 2021; Münstedt, 2022; Rahim et al., 2017; Shamsudin et al., 2019; Toledo-Hernández et al., 2022). Moreover, the study protocol highlighting the randomisation process and blinding should be further detailed in the methodology to prevent the matter of bias and enhance the reliability of the outcomes. The usage of raw honey without sterilisation is also a cause for worry as the antibacterial properties of honey could be compromised if the honey is not medical-grade. Sterilisation with Gamma radiation is crucial to kill bacterial spores present in the honey which could create cross-contamination to the wound. However, there is no standardisation for sterilisation in clinical studies as sterile and unsterile honey produced positive outcomes in wound healing as demonstrated in the literature review. As per the 10 studies reviewed, only one study (Imran et al., 2015) mentioned the use of sterile honey but there was no mention of sterile honey in most of the studies. Therefore, the importance of sterilisation and the standardisation of application was not discussed in detail in other studies in the literature review. Manuka honey-impregnated dressings used in the studies could be sterilised by Gamma irradiation but not mentioned. Therefore, unstandardised honey and application may be a limitation to improve healing outcomes of wounds but yet to be ascertained to date (Tashkandi, 2021). Therefore, there may be a factor of bias and inconsistency in evaluating the efficacy of honey in DFU. Unstandardised honey

application in wounds for research purposes or clinical settings should be addressed in future studies to produce consistent outcomes.

Another factor that could have contributed to the risk of bias is the inconsistent grade of ulcer in the study protocol. The Wagner grade of ulcers also differed across the studies, namely grades 1-2 (Bashir et al., 2018), grades 1-4 (Jan et al., 2012), grade 2 (Agarwal et al., 2015), and grades 2-4 (Al Saeed, 2019). In the study by Karimi et al. (2019), different Wagner grades were mentioned in the honey group and olive oil group but not described in detail. Different grade of ulcer was included among the three groups (nanocrystalline silver Ag, manuka honey, and conventional dressing) but not elaborated by Tsang et al. (2017) as well. Similarly, Koujalagi et al. (2020) did not mention the grade of ulcer included in his study. Different grades and types of honey could be independent risk factors for the healing rate of DFU as a higher Wagner grade of more than 3 has a lower healing rate (Monteiro-Soares et al., 2014; Örneholm et al., 2015). In view of the differences in the grade of ulcer, it was difficult to confirm and generalise the findings, ultimately to draw a conclusion.

Another limitation of the literature review was the measurement tool. Different tools were used in studies that measured the size of ulcers. Conventional measuring tools such as a ruler, measuring tape, and graph paper were used in the studies discussed in the literature review. While studies by Kamaratos et al. (2014) and Imran et al. (2015) used the ruler technique, Koujalagi et al. (2020) and Bashir et al. (2018) used transparent graph paper and measuring tape, respectively. Ruler measurement is the easiest to perform while it can be quite cumbersome to use graph paper and measuring tape. But among the three, transparent graph paper is more accurate than a ruler and measuring tape. Likewise, it is difficult to measure cavity wounds with all three methods. Therefore, there may be inconsistency in measurement across the studies, which could affect the healing outcomes. The utilisation of advanced validated measurement tools is highly recommended to produce a precise wound measurement. However, there is a probable cause that the researchers were not able to advance measurement methods during that point in time. However, the other studies used more updated measurement tools to measure the size of ulcers at different timelines, such as digital photography (Al Saeed, 2019) and a digital wound measurement device known

as Visitrak (Tsang et al., 2017). Despite using a more prominent measurement tool, the procedure to take the wound pictures was more time-consuming as the angle of the photo taken should be at a certain distance and consistently followed in every measurement. There was a lack of details describing how the measurement was done in the studies with the digitalised system. The validity and reliability were also not discussed in the publication of the research. The conventional method invites cross-contaminations compared to digital methods. Therefore, selecting the appropriate validated reliable measurement tools is undeniably crucial to ensure the findings are non-contact measurement, reliable, and consistent (Goldman & Salcido, 2002; Wang et al., 2017). Given different measurement tools were used in the above-mentioned studies, the results may need more explorations with a more precise tool to confirm their findings.

As mentioned in numerous studies, there are various ways to evaluate the healing outcome for DFU which is very much research-dependent. Based on the literature review, the primary outcomes of many studies were the time to healing, complete closure, ulcer size reduction rate, healing rate, hospitalisation rate, infection reduction rate, antibiotic-free days, and wound healing score. Given the various healing outcomes of the studies and different types of honey used in the literature review, it may not be feasible to conclude the findings because the aims of the studies were different therefore the study protocol was not consistent except for Tsang et al. (2017) and Koujalagi et al. (2020) who used percentage size reduction as primary healing outcome. The rest of the studies evaluated different healing outcomes. For example, Bashir et al. (2018) used the time to achieve granulation and epithelisation as the primary outcome whereas the primary outcome of Al Saeed (2019) was an infection-free rate, complete healing, and hospitalisation days. Time to healing meant the appearance of granulation tissue for Bashir et al. (2018) whereas it meant ready for skin grafting or surgical closure for Agarwal et al. (2015). All studies in the literature review provided different descriptions of healing outcomes in the treatment of DFU. Hence, the definition of healing outcome varies from one study to the other in the literature review. Given the fact that a very small number of studies evaluated the efficacy of honey in DFU based on different healing outcomes, the findings in the literature may not be conclusively confirmed.

The age factor has been a matter of controversy in the healing of DFU for decades. However, it is a general understanding that the healing outcome in DFU is reduced with the ageing population above 60 years old (Bonifant & Holloway, 2019). Thus, age can be considered as one of the risk factors for the healing of DFU. In the literature review, various age groups were included in the studies, namely 54 years old (Imran et al., 2015), 52 years old (Agarwal et al., 2015), 65 years old (Al Saeed, 2019; Chauhan et al., 2020; Jan et al., 2012; Karimi et al., 2019; Tsang et al., 2017), and 45 years old (Bashir et al., 2018), while some studies did not mention the age of their participants (Kamaratos et al., 2014; Koujalagi et al., 2020). It is baffling to observe that the older diabetic population in Al Saeed (2019) demonstrated a higher healing time with manuka honey as compared to silver dressing; whereas, a similar age group in Tsang et al. (2017) showed less efficacy of honey as compared to nano-crystallised silver. Similarly, a younger population of about 45 years old in Bashir et al. (2018) indicated that honey was less effective than negative pressure vacuum therapy. For the remaining studies, the healing outcome was more prominent with honey irrespective of the age factor. Therefore, the findings in the literature review may contradict the earlier findings that ageing affects healing. However, as the studies are limited in numbers, the inconsistent findings require further investigations to determine if age affects the healing outcome in the treatment of DFU with honey. A longer duration of the study with the segmentation of age groups and a bigger sample size may provide the answer to this methodological limitation. However, age should be combined with gender factors to conclude the findings.

The study setting was also another inconsistent factor in the literature review. Some participants were treated in the outpatient clinic while others were admitted. Generally, those who were admitted stayed in a controlled environment given care, treatment, and diet as compared to admitted patients. Similarly, the site of the ulcer and the number of ulcers. The studies did not mention whether there was more than one wound on each foot and the location of the ulcer. With the different ulcer characteristics among the participants in the literature, wound healing outcomes could be affected if there were multiple ulcers on the same foot, Site of treatment also was different in the literature review. For example, participants with negative -pressure vacuum therapy was kept in the ward while the other participant with honey treatment stayed home and came for follow-up every other day

(Bashir et al., 2018). Therefore, healing outcomes could have a risk of bias as different sites may produce different healing outcomes. The site of the ulcer also could make a difference in the healing outcome of DFU. In the literature review, the exact location of the ulcer was not stated except for which limb either right of left stated in the study protocol. Wounds on the plantar heal slower than the dorsal wounds. Despite the studies in the literature review, it may not be feasible to confirm the findings because of the lack of details on the study protocol.

2.11 SUMMARY OF RESEARCH GAP

Based on the recommendation of IWGDF guidelines, the most effective wound dressing should be able to provide a moist environment, reduce the bioburden, be impermeable to microbial, and gaseous exchange, and stimulate healing. Other benefits should include de-sloughing, removal of exudate, painless dressing removal, and cost-effectiveness (Bakker et al., 2016; Game, Apelqvist, et al., 2016; Hinchliffe et al., 2020). To date, no one wound dressing could provide optimal efficacy, especially in overcoming infection and antibiotic resistance in DFU. Given the therapeutic properties of honey, it fits the bill because it has antibacterial, anti-inflammatory, and antioxidant properties to provide moist wound healing and expedite healing (Yildiz Karadeniz et al., 2023; Wu et al., 2015). The majority of the reviewed studies demonstrated that honey was more effective compared to conventional dressings in the treatment of DFU except for comparison with advanced dressing like negative pressure vacuum therapy and nano-crystallised silver dressing. Despite the limited number of studies with honey related to the healing of DFU in the last decade, the healing outcomes were shown to be promising due to its antibacterial, anti-inflammatory, and antioxidant properties. Faster healing time with wound size reduction, bacterial clearance, and improved healing rate as revealed in the literature review could be advantageous in the treatment of DFUs. Based on the findings, honey has a high potential to be an effective wound dressing to resolve antibiotic resistance, fight infection, stimulate wound healing, and expedite the healing process of DFU due to its therapeutic properties. The positive

healing outcomes with honey could add value to the healing of DFU as diabetes-based foot complications, infection, gangrene, and lower limb amputations are on the rise.

Although much importance has been given to manuka honey or honey produced by honey bees in general, the efficacy of kelulut honey produced by stingless bees is yet to be explored for wound healing in DFU. Kelulut honey can be explored to further improve wound healing outcomes due to its anti-oxidant potency as compared to manuka honey (Abdul Karim & Anum, 2019; Maringgal et al., 2019; Yaacob et al., 2017). Given the methodological differences between the studies in the literature review, the findings may not be conclusive to confirm that honey has significantly superior efficacy than conventional dressing in the treatment of DFU. The limitations and inconsistencies in the study protocol, measurement tool, type of honey used, and small sample size based on the literature review could be a hindrance to achieving statistical significance in clinical studies with honey in the treatment of DFU. Hence, the limitations need to be addressed to determine the efficacy of honey in the treatment of DFU. Given that no studies to date have compared kelulut honey produced by stingless bees *Trigona sp* and honey from honey bees *Apis sp*, or manuka honey, the exploration into this subject matter is timely. Furthermore, clinical evidence showing the associated factors influencing the healing outcome of DFU, which is deemed important to identify the independent risk factors for DFU in honey-related clinical studies was also less in common based on the literature review. Therefore, the efficacy of comparison studies between kelulut honey and manuka honey, and the associated factors that can influence the healing outcomes of DFU with honey dressing need to be investigated to find an effective pathway to accelerate the healing of DFU.

2.12 CHAPTER SUMMARY

The management of DFU has certainly experienced drawbacks in recent times as the treatments were not able to achieve targeted wound healing outcomes as DFU can be easily infected and resistant to antibiotics. The healing process of DFU tends to be prolonged and foot complications are inevitable. The application of honey-based wound dressing has

certainly improved the healing outcomes to a certain extent due to its antibacterial, anti-inflammatory, and antioxidant properties as demonstrated in the literature review. However, significant outcomes related to the efficacy of honey in the treatment of DFU remain less in number. The literature review has highlighted the strengths and the weaknesses of the studies that showed further investigations are needed to strengthen the clinical evidence on the efficacy of honey in the treatment of DFU. The limitations in the reviewed studies further highlighted the lack of high-quality study design to illustrate the efficacy of honey in the treatment of DFU. Similarly, the literature search has highlighted the absence of studies related to stingless bee kelulut honey in wound healing and comparative studies between different types of honey in the treatment of DFU. Thus, the insights provided by the literature review need to be addressed to determine the efficacy of stingless bee kelulut honey using the local species *Trigona itama* which is less explored in wound healing as compared to medical-grade manuka honey (*Apis mellifera*).

CHAPTER THREE

METHODOLOGY

3.1 INTRODUCTION

The objective of this chapter is to elaborate on the methods used to conduct the present study. It begins by outlining the research questions followed by the research design, site selection, selection of the research population, sample size calculation, recruitment criteria, and data collection procedure. The methodology devised for the study was based on previous studies on the efficacy of honey in the treatment of DFU due to the inexistence of references related to the efficacy of kelulut honey in wound healing. Finally, the chapter concludes with ethical considerations and statistical methods for data analysis.

3.2 RESEARCH QUESTIONS

The main objective of this study was to evaluate the effectiveness of kelulut honey in the treatment of DFU. The research design was based on the following research questions:

1. How is the efficacy of kelulut honey as compared to medical-grade manuka honey following MDT in the treatment of DFU based on the percentage of ulcer size reduction from baseline to day 7 and day 14?

2. Is there any relationship between the participants' socio-demography (age, gender, BMI), clinical characteristics (duration of diabetes, HbA1c, CKD), ulcer characteristics (grade, size, site, duration of ulcer), and the percentage of ulcer size reduction?

3.3 RESEARCH DESIGN

The present study was conducted during the COVID-19 pandemic when the focus was on managing the positive-tested patients, and a limited number of patients with non-COVID-19 conditions were allowed to seek treatment at outpatient clinics in UMMC. During the pandemic, patients with DFU were referred to the peripheral health clinics as the wound management team was shorthanded and the wards were overflowing with COVID-19 admissions. Thus, recruiting participants for the present study was challenging and it was not feasible to have a longer observation period. The researcher and the study team had to work with a minimum number of patients who were tested negative for COVID-19 and observed for a shorter duration to avoid a high number of dropouts during the point of time. Hence, the quasi-experimental study design was selected and deemed appropriate for the present study as true randomisation and double-blinding were not feasible then. Furthermore, the quasi-experimental approach was reported to be a pragmatic choice to evaluate real-world intervention and not research-laboratory intervention as highlighted by Schweizer et al., 2016).

3.4 STUDY SETTING

University Malaya Medical Centre (UMMC) was selected as the research setting of the present study because it represented a descriptive healthcare environment where the applicability of the findings could be possible, ethics oversight, and availability of appropriate resources. Various socio-economic backgrounds of patients with DFU seeking treatment at UMMC represented the cross-segmentation of the population in Klang Valley. This could help in the generalisability of findings in this study. Historically, UMMC is the oldest and largest tertiary teaching hospital in Malaysia where it started as the teaching hospital for the Faculty of Medicine, University of Malaya in 1866. UMMC is located at the Kuala Lumpur-Selangor border and is a referral site for peripheral health clinics and district hospitals. The orthopedic team was in charge of DFU in UMMC and has prior experience in using alternative treatments such as medical-grade manuka honey and MDT

in wound healing. Therefore, it was rational, counterproductive, and pragmatic to select UMMC as the study setting.

3.5 STUDY POPULATION

Sample, commonly known as the study population, refers to participants who meet the recruitment criteria and who represent the cross-segmentation of the targeted population. The present study used the most common, consecutive sampling technique for the recruitment of participants (Polit and Beck., 2017). This technique can be easily utilised to continuously recruit participants using the inclusion and exclusion criteria to fulfil the targeted sample size in the present study. In addition, the consecutive sampling technique could control the factor of bias in the selection of participants (Thewes et al., 2018). Socio-demographic factors such as age, gender, and duration of diabetes have been evaluated in several studies as risk factors for the healing of DFU. The outcomes of the evaluations on the mentioned risk factors were inconsistent. Hence, more evaluations are required to confirm the association between age, gender, and duration of diabetes with the healing outcome in DFU (Al-Rubeaan et al., 2015; Jiang et al., 2015; Moura et al., 2013; Parisi et al., 2016; Wang., 2014; Wukich et al., 2018). Previous studies also evaluated the clinical characteristics that include CKD, BMI, and HbA1c; however, the findings remain inconclusive to date. Nevertheless, clinical characteristics such as BMI, HbA1c, and CKD have been identified as prognostic factors that can influence the healing outcome of DFU (Dugbartey et al., 2022; Lin et al., 2020; Rosedi et al., 2022). Therefore, the clinical characteristics of the study population were included in the baseline characteristics.

3.5.1 Inclusion and Exclusion Criteria

The research population of the present study consisted of 60 adult participants with no restriction of age, type 2 diabetes (females and males) with sloughy DFU Wagner grade 2-3 of more than 2 weeks. Their blood test results were referred to before the recruitment

process. Participants with hemoglobin (Hb) > 10g/dl, normal ankle brachial index (ABI) > 0.8, culture result with common pathogen non-multidrug resistant organism (MDRO) or methicillin-resistant *Staph aureus* (MRSA), and HbA1c < 9 mmol/l who were presented to the outpatient orthopedic clinic in UMMC between December 2021 till October 2022 were recruited for this study. Their recruitment was based on the inclusion and exclusion criteria. All 60 participants who consented to be treated with MDT for debridement purposes followed by honey treatment were enrolled in the study. The participants were divided equally into two groups with 30 participants in the intervention group were treated with kelulut honey and another 30 in the control group were treated with medical-grade manuka honey. The exclusion criteria were DFU (Wagner grade 4-5), venous ulcers, ischemic painful ulcers, malignant ulcers, anemia, duration of ulcer > 1 year, those with multiple ulcers per foot, life-threatening infection or sepsis, end-stage kidney failure or dialysis, entomophobia (fear of insects), and known allergy to antibiotics, honey or bee products. The inclusion criteria for the selection of participants are summarised in Table 8.

Table 8: Inclusion Criteria for the Selection of Study Population

Inclusion Criteria
Malaysian Adult Diabetic > 18 years old
Sloughy diabetes-based foot ulcer
2 weeks <DFU< 1year
Amputation possibly deferred
DFU classification: Wagner grade 2, 3
Type 2 diabetes
HbA1c < 9
Hb > 10

Ankle-brachial pressure index (ABPI) >0.8 (no peripheral arterial disease)
Chronic kidney disease (CKD) ≤ stage 3
Patients with hemoglobin, Hb > 10 g/dl
Not more than 1 DFU per foot
No known allergy to antibiotics, honey or bee products
Keen on both honey and MDT
Common pathogen non-multidrug resistant organism (MDRO) or methicillin-resistant *Staph aureus* (MRSA)

Based on the inclusion and exclusion criteria, the selection of participants was undertaken in the orthopedic outpatient clinic by the researcher and study team. The participants were divided into two groups (intervention and control group) by a simple sampling technique. Random numbers (1 and 2) with equal probabilities were placed in a bowl and drawn by the participants. Those with number 1 were assigned to the intervention group (Group 1) and number 2 to the control group (Group 2). Despite not going through a complete randomisation process as the present study is quasi-experimental, the simple randomisation technique could enhance the quality of the outcome and reduce the risk of bias. To further strengthen the study protocol, single-blinding was implemented where the participants would not know the type of honey applied to their wounds. However, the study team who were involved in the application of honey dressing during the study period was aware of the type of honey applied.

The participants were briefed about the study protocol and information on MDT and honey based on the information sheet approved by the Ethics Committee. Informed consent forms were given for the participants to sign as it indicated that they were aware of the treatment procedure, aware of any risk involved, and agreed to participate. The information sheet was given to each participant or family member to further understand the treatment involved. The participants were given adequate time and space to decide on their participation without fear or favor. Consented participants were given assurance that

information confidentiality would be always maintained. The contact number of the researcher and principal investigator as stated in the information sheet were made known to the participants to communicate if there were any clarification required.

3.6 SAMPLE SIZE

Sample size refers to the required number of participants in the study population based on the aim of the study. As with any other clinical study, sample size plays a huge role in determining the significance of the outcomes.

3.6.1 Sample Size Calculation

Sample size calculation is important in research as it enhances reliability and provides a generalisation of the outcomes. To fulfil the aim of this study, the calculation of sample size relied on producing a higher probability of achieving the targeted outcome, at least a minimum 20% reduction of size in the DFUs. The minimum detectable size changes in DFU were adopted to obtain an adequate number of participants with a higher probability. The calculation was based on similar interventional studies with a minimum detectable effect size as mentioned in similar interventional studies in DFU (Purnama et al., 2021; Xu Tian et al., 2014a; Wang et al., 2019; Yildiz Karadeniz & Kaplan Serin, 2023).

The calculation showed a minimum of 55 samples required to determine the statistical significance in the outcome of the study in each group with a standard deviation of 37.5%, an error estimate of 5%, and an 80% probability of detecting changes in the size of ulcer. Due to the COVID-19 pandemic, a bigger sample size was not feasible due to the minimisation of physical contact between patients, the study team, and the researcher. Therefore, 60 participants were recruited in consideration of the possible 10% dropout during the data collection process.

The formula for sample size calculation (Cornish, 2006):

$$n = \frac{2(Z_a + Z_{1-\beta})^{2\sigma 2.}}{\Delta^2} = 55$$

Std deviation = 0.375 $Z_{1-\beta} = 0.8416$ $Z_a = 1.96$ $\Delta = 0.2$

3.7 DATA COLLECTION

3.7.1 Participants' Socio-Demography and Clinical Data

Socio-demographic data was collected from the participants pertaining to their age, gender, ulcer duration, duration of diabetes, and past treatment history. Measurement of height and weight at the outpatient treatment room was used to manually calculate the body mass index (BMI) for each participant. Other information on CKD, HbA1c, and Hb were retrieved from the UMMC patient database by the principal investigator. Clinical assessment of the ulcer was targeted on the duration, site, size, and grading of the ulcer. The participants' socio-demography and clinical data was documented with the size of the ulcer measured at baseline followed by on day 7 and 14 to fulfil the objectives of this study.

3.7.1.1 BMI

BMI was calculated based on the participants' weight and height (kg/m^2). Generally, BMI is age-dependent for both male and female. A normal or healthy BMI is between 18.5 to 24.9 while a BMI of 25 to 29.9 is categorised as overweight, a BMI above 30 falls under the obese category, and a BMI below 18.5 is considered underweight. It was reported that any increase in BMI increases the risk of foot complications among diabetics (Garber et

al., 2017; Yazdanpanah, Shahbazian, Nazari, Arti, et al., 2018). The BMI categories based on WHO classification are summarised in Table 9.

Table 9: Classifications of BMI

Classification	BMI	Risk of Comorbidities
Underweight	<18.5	Low
Normal	18.5-24.9	Average
Overweight	25-29.9	Mildly elevated
Obese	>30	Moderate to high

3.7.1.2 CKD

ElSayed et al. (2022) defined CKD as a gradual decrease or loss of kidney function over time. It refers to a continuous kidney function loss in three months or more with an estimated glomerular filtration rate (eGFR) of less than 60 ml/min per 1.73 square metres. The kidneys get damaged and unable to filter blood as they should and the condition becomes “chronic” since the damage happens slowly over a period (Vaidya and Aeddula, 2022). This damage causes waste to build up and accumulate in the body, causing chronic heart disease and stroke, and if left untreated, patients will end up with kidney failure which requires dialysis. One of the reasons for CKD is poorly controlled diabetes. Based on the American Diabetes Association Guideline for Diabetes Standard Care 2023 report, CKD stage 1 refers to eGFR 90 or higher (normal), CKD stage 2 refers to mild damage, CKD stage 3 refers to moderate kidney damage, and CKD exceeding stage 3 refer to severe kidney damage (Dugbartey et al., 2022). The stages of kidney disease guidelines are described in Table 10.

Table 10: Stages of Kidney Disease

Stages of CKD	eGFR	Description
1	90 or higher	Kidney works as normal
2	60-89	Mild kidney damage
3a	45-59	Mild to moderate kidney damage
3b	30-44	Moderate to severe kidney damage
4	15-29	Severe kidney damage
5	Less than 15	Most severe established renal failure. Requires dialysis (end-stage renal disease)

3.7.1.3 HbA1c

Based on the guideline set by WHO, HbA1c of 6.5% is a standard to determine the diagnosis of diabetes. The control of diabetes is based on the level of HbA1c which is defined as average blood glucose level over the past three months. The levels of HbA1c and the status of glucose control (WHO, 2015) are illustrated in Table 11.

Table 11: HbA1c Levels and Status of Glucose Control

HbA1c Value (%)	Status of Glucose Control
Less than 5.7	Non-diabetes range
5.7 – 6.4	Indicative of pre-diabetes
More than 6.5	Diabetic
6.5 – 7.0	Good control of diabetes
7.1 – 8.0	Fair control of diabetes
Above 8.0	Poor control of diabetes

3.7.2 Wound Characteristics

The participants' DFUs were subjected to clinical assessment which included the size, grade, and duration of ulcer. Past clinical evidence indicated that wound characteristics play a substantial role in predicting the healing outcomes of DFU (Monteiro-Soares et al., 2020; Smith-Strøm et al., 2017). Therefore, these wound characteristics were documented at baseline and the size of the ulcer continued to be documented on days 7 and 14. Concerning the correlation of socio-demography and clinical characteristics with the trajectory of healing, the characteristics of DFU itself could be a determinant factor for wound healing (Deribe, 2014; Kee et al., 2019; Rosedi et al., 2022).

3.7.2.1 Classifications of DFU

Classifications of DFU were formulated by a group of experts in the field of chronic wound management which became a focal point in predicting healing outcomes in clinical settings and research (Bus et al., 2016; Fife et al., 2016; Lipsky et al., 2016; Markakis et al., 2016).

Based on the guidelines recommended by IWGDF (2019), the most common externally validated classifications of DFU were Wagner, University of Texas, PEDIS (Perfusion, Extent, Depth, Infection, Sensation), and SINBAD (Site, Ischemia, Neuropathy, Bacterial infection, Depth). Nevertheless, the Wagner, SINBAD, and University of Texas classifications were highly recommended and widely recognised for the evaluations of DFU in research and healthcare settings (Game et al., 2016; Jeon et al., 2017; Monteiro-Soares et al., 2014; Pickwell et al., 2015; Vibha et al., 2018).

The Wagner classification system for DFU was used in the present study to predict healing outcomes as diabetics with severe ulceration had a high probability of undergoing lower limb or digit amputations. According to the Wagner classification for DFU, Grade 0 is defined as no ulcer with intact skin, Grade 1 describes a superficial ulcer with full skin thickness, Grade 2 describes ulcer extending to ligament and muscle (no bone involved), Grade 3 describes deep ulcer with cellulitis or abscess, Grade 4 localised forefoot gangrene (toe, heel), and Grade 5 describes extensive gangrene involving the whole foot which may end up with amputation (Shah et al., 2022). Wagner's classification for DFU is summarised in Table 12.

Table 12: Wagner Classification Grading System for DFU

Grade	Description
Grade 0	No ulcer. Intact skin but high-risk foot.
Grade 1	Superficial ulcer with full skin thickness.
Grade 2	Ulcer extending to ligament and muscle (no bone involvement).
Grade 3	Deep ulcer with cellulitis or abscess with bone involvement.
Grade 4	Localised forefoot gangrene (toe, heel).

Grade 5	Extensive gangrene (whole foot).

3.7.2.2 Site of DFU

Another wound characteristic that could be a predominant factor in determining the healing rate of DFU is the site of the ulcer. Several studies have indicated that the site of ulcer could influence healing outcomes in DFU. For instance, it was revealed that ulcers at the plantar region have a decreased healing rate as compared to the dorsum (Jeon et al., 2017; Monteiro-Soares et al., 2014; Noor et al., 2015). However, the outcomes were not conclusive in establishing the relationship between the site of ulcer and the healing rate of DFUs. Hence, this revelation related to the site of ulcer influencing the healing outcome in DFU requires further confirmation (Abd Wahab et al., 2015; Everett & Mathioudakis, 2018; Pemayun et al., 2015). Therefore, the site of ulcer was included as one of the wound characteristics in the present study to assess its possible influence on the healing outcome of DFU when treated with honey. In this study, the site of ulcer was categorised into 5 regions of the foot as shown in Table 13.

Table 13: Site of Ulcer Categories

Category	Site of ulcer
1	Plantar
2	Dorsum
3	Forefoot/Toes
4	Lateral
5	Hindfoot/Heel

3.7.2.3 Duration of DFU

According to Smith-Strøm et al. (2017), the duration of DFU could be a risk factor for DFU and increase the probabilities of infection, gangrene, and even amputation. A multi-centered observational study by Ugwu et al. (2019) denoted that the duration of ulceration of more than a month is a strong predictor for lower-limb amputations. Duration of DFU was defined as the time from patient-reported ulcers to the beginning of treatment initiation. In the present study, participants with an ulcer history of more than 2 weeks were included whereas those with ulcers of more than 1 year were excluded.

3.7.2.4 Size of DFU

Jenkins et al. (2019) mentioned that the projection of healing in DFU was expected to correlate with the size of the ulcer depending on the timeline of observation. Hence, the size factor had been an imminent baseline characteristic of concern in interventional studies. Previous studies highlighted the size of ulcers < 5 cm^2 and more than 5 cm^2 demonstrated differences in the healing rate of DFU but the differences were not statistically significant. However, the correlation between size and healing rate of DFU has seen much debate of late but remained inconclusive as there are other associated factors such as the site of ulcer, grade, and duration of ulcer could contribute to the effect of size in the healing of DFU (Venkataramana et al., 2020; Wang et al., 2019; Yasin et al., 2018). Based on the research question for the present study, the efficacy of kelulut honey and medical-grade manuka honey was evaluated based on the ulcer size reduction from baseline to days 7 and 14. The measurement of ulcer size required a reliable and validated digital instrument as described in the next segment.

3.7.3 Instrument for Wound Measurement

The healing outcome of DFU could be measured via many methods and it is undeniably important that the accuracy of ulcer size could be a predominant factor to influence healing.

Changes in size were calculated by clinicians to determine the healing rate and chart the healing trajectory for DFU. One of the conventional and most common tools used to measure the size of wounds is a ruler while other possible tools include graph paper and measuring tape. However, these methods lack precision and accuracy (Gethin et al., 2015; Gottrup & Apelqvist, 2012; Tallis et al., 2013). As technology advanced, tools to measure the size of wounds evolved to a more digitalised approach with higher accuracy and precision that included a mobile wound monitoring application. In recent times, several publications highlighted the use of wound photography as a reliable tool for wound measurement. The latest technology in wound measurement does have its limitations including the availability of internet connection, subjective assessment by clinicians, no definitive wound edge measurement, and sharpness of images but the precision outweighs the disadvantageous (Barakat-Johnson et al., 2022; Jørgensen et al., 2016). Therefore, digital application software was the instrument used to calculate the size of ulcer at baseline, day 7, and day 14 in this study.

3.7.3.1 Wound Measurement

Digitalisation of wound applications has certainly improved the precision of wound documentation and improved patient outcomes. In the present study, NDKare™ mobile wound monitoring application was used to measure the size of DFU continuously from baseline to days 7 and 14. NDKare™ is validated, FDA-approved, CE-marked, and registered with the Health Sciences of Singapore (HSA) for the documentation of wound progress and to formulate strategies that can expedite the healing of chronic wounds. Past reports denoted that the usage of NDKare™ can reduce the time taken for measuring wounds by 80% and improve the rate of healing for chronic wounds by 11%. Accurate measurement of size, granulation, slough, and necrosis with NDKare™ provides clinicians with useful information on wound progress and assists them in efficiently preparing the wound for other interventions. It is a user-friendly system that can be operated online and offline. It used advanced image processing technology which showed the wound measurement speedily and accurately (Kuang et al, 2021). Healthcare professionals who handle chronic wounds could be securely connected with one platform using the NDKare™

app and it is free of charge. Few users could share and upload wound pictures into the system database using their smartphones. Therefore, assessing information on the wound progress by more than one healthcare personnel could be advantageous in expediting decision-making on the next course of treatment to manage, improve healing, and reduce foot complications (Marimuthu & Makhtar, 2020). The potential benefit of the NDKare™ app was evaluated at Kuala Lumpur General Hospital (Nair, 2018). Information regarding NDKare™ is attached in Appendix B.

The NDKare™ app was indicated to save wound measurement time, reliable, and has higher accuracy in wound assessment and monitoring. In research, the use of NDKare™ app may replace the old measuring technique using rulers which may not be accurate and efficient in wound progress documentation. For this study, the application was initiated by downloading, and installing the NDKare™ app. Specific passwords were created for the researcher, doctors, and the principal investigator in the study team that monitors the treatment progress of wounds. Special stickers supplied by the supplier were kept in the locked cabinet in the treatment room that was to be placed beside the wound as a reference before wound images were captured with the smartphone which was directly linked to the database in the cloud. The wound images would be uploaded and sent instantly to the database. Information about the size of ulcer was stored in the NDKare™ app database and could be retrieved by the principal investigator and researcher. Based on the differences in size before and after interventions over a period, time to healing could indicate the effect of the intervention, progress of treatment, and chart the healing trajectory of DFU. In a recent study, the accuracy and practicality of NDKare™ were shown to provide a consistent measurement of DFU. Hence, NDKare™ was recommended as a fast, accessible, and user-friendly measurement tool that could be absorbed into the management of wounds (Reifs et al., 2023).

Apart from accuracy, another important rationalisation of using this software was the non-contact approaches taken during data collection in UMMC due to the COVID-19 pandemic. COVID-19 caused many disruptions in the care of patients, especially for diabetic individuals with foot ulcerations or complications. During the pandemic, one of the major concerns of the orthopaedic team was to reduce the contact between the study

team and participants during data collection. As COVID-19 reshaped the world, advanced wound assessment applications were deemed most appropriate (Barakat-Johnson et al., 2022). Therefore, the use of NDKare™ is crucial and timely for patient management during the COVID-19 pandemic to ensure contactless exposure. The contactless system software allowed wound images to be captured, stored, and analysed using the smartphone anywhere, anytime with much less time. The NDKare™ system is an efficient non-contact method to measure the wound size of DFUs. This app is considered a viable option for wound measurement and was used throughout the present study to measure the size of ulcers in both intervention and control groups at baseline, day 7, and day 14. The visual image of the digital mobile application of NDKare™ is shown in Figure 7.

Figure 7: Image of the Digital Mobile Application of NDKare™

3.8 RECRUITMENT AND TRAINING FOR THE STUDY TEAM

Upon the recommendation by the principal investigator, 3 staff nurses who were trained in wound management were recruited into the study team. They were tasked to assist in managing the appointments for the follow-ups of participants in the orthopaedic treatment room and to assist in the application of honey during the study period. The training for the

staff nurses was conducted online with an emphasis on the overall study objectives and study protocol for data collection. They were also trained on the MDT and honey application procedure using PowerPoint slides. Two orthopaedic medical officers were tasked to handle the recruitment of participants and monitor the overall health condition of the participants throughout the present study. They were also briefed on the selection criteria and trained on using the wound measurement tool NDKare^{TM} as they might need to capture the wound images at baseline, day 7, and day 14.

3.9 TREATMENT PROTOCOL

The treatment protocol was divided into two phases whereby Phase 1 included MDT for debridement and Phase 2 involved the application of honey according to the designated groups.

3.9.1 Phase 1: Debridement

The baseline characteristics of the study population (60 participants) and the clinical characteristics of DFU were documented before the commencement of Phase 1 except for the size of the ulcer. The debridement of the DFUs in the study population was performed using MDT. Sterile maggots were ordered from the Institute of Medical Research, Kuala Lumpur (IMR) where the colony of *Lucilia cuprina* was kept in a controlled environment. Sterilisation of the eggs was done at IMR using a patented method and the larvae hatched from the eggs were collected with a special scoop and transferred into a sterile vial. Each vial could accommodate 100–250 sterile maggots. The vials were packed in a cooler box together with plastic sterile forceps and delivered to UMMC for application. Once the maggots reached the hospital, the participants' wounds were cleaned with normal saline aseptically. The maggots were prepared by mixing them in the vials with normal saline, collected using the sterile pipette, and transferred onto sterile gauze. The sterile gauze with maggots was placed on the wounds and covered with a sterile gamgee as primary dressing and the edges were sealed using hyperfix tape which would serve as a "cage" to contain the

maggots. The dressing was further strengthened with secondary dressing using another gamgee and bandaged. The maggots were applied to the wound soonest possible since the timeline for the life of the maggots was 24 hours. As the final step, the date of application was written on the bandage and noted in the patient information chart. The dressings were opened after 72 hours and the removal of maggots was conducted. A second application was decided if complete debridement was not achieved in the first application. As shown in the previous study, it took 1 to 2 cycles of 200 maggots to achieve complete debridement within 3-6 days on average (Marimuthu & Makhtar, 2020). The image for MDT application process, the illustration of maggots on the wound during application and after 72 hours is attached in Appendix C. Once the wounds achieved complete debridement, the study population followed through Phase 2 for honey application. Flowchart for MDT is illustrated below in Figure 8

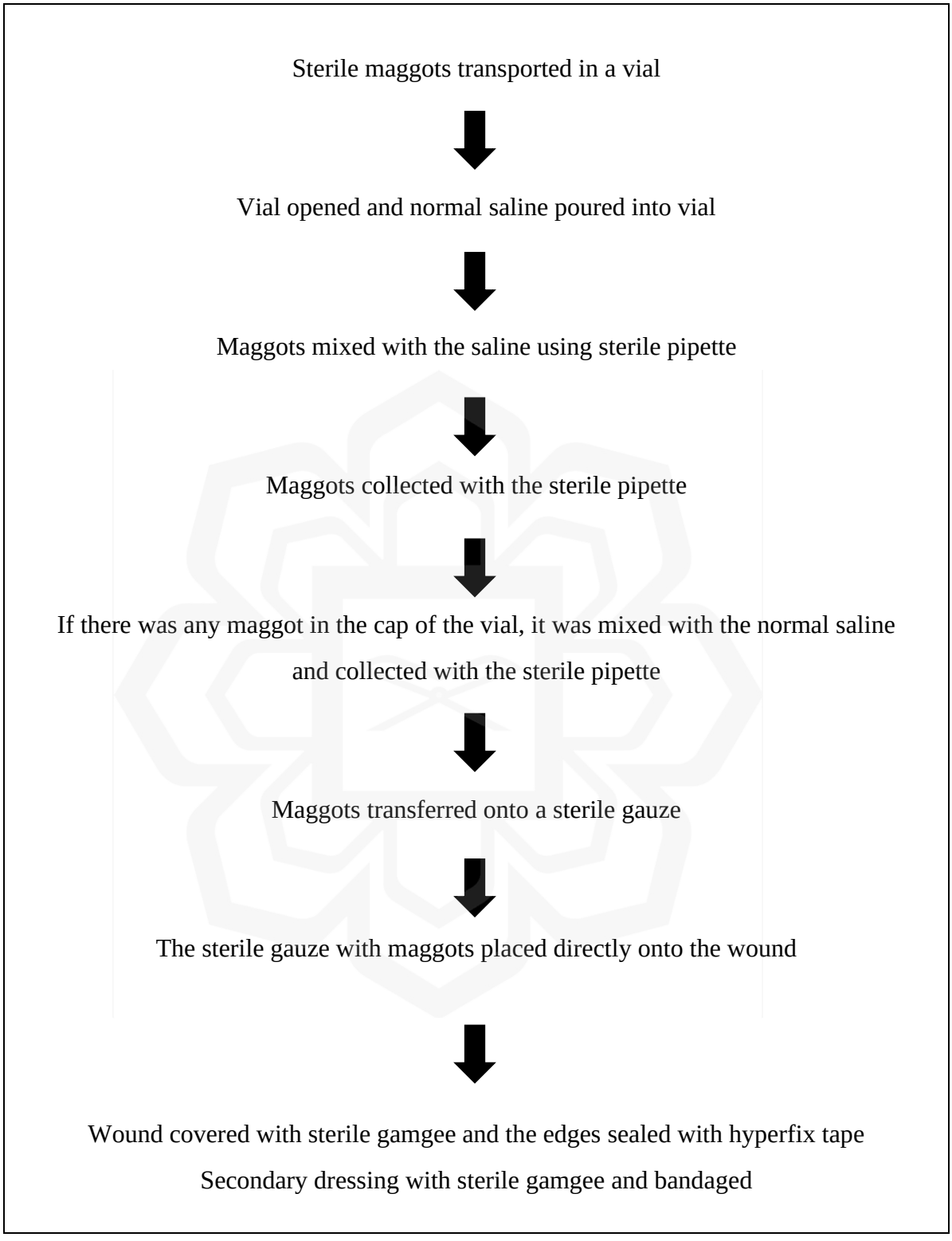

Figure 8: Flow chart for MDT application

3.9.1.1 Ambulatory Protocol for MDT

Upon application of MDT, participants were required to minimise mobilisation until the dressing opened in 3 days or 72 hours. Furthermore, the participants were advised not to wet their feet, and if possible, to rest in bed. If the DFU were located at the plantar aspect, participants were advised not to step down on the floor to avoid damaging the sterile maggots. Within the 3 days, dark staining and wetness on the bandage could be observed by the participants and ambulation could increase the wetness and leak in the bandage. Thus, the dressing could be required to be opened earlier than scheduled which could affect the outcome of debridement by the maggots. In short, dressing with MDT should be preserved with minimal ambulation. For wounds located at plantar regions, extra gamgee were applied to offload the pressure and prevent damage to the maggots. Minimal ambulation reinforced by the researcher to the participants.

3.9.2 Phase 2: Intervention

Upon completing Phase 1, the study population proceeded to Phase 2 where they were divided into two groups with 30 participants in each group: Group 1 (intervention group treated with kelulut honey) and Group 2 (control group treated with medical-grade manuka honey). At this point, the ulcer size measurement was performed using NDKare^{TM} before the honey application. For this study, medical-grade manuka honey was used as the control because this type of honey dressing was already in use for the treatment of DFUs in UMMC and other hospitals in Malaysia.

3.9.2.1 Kelulut Honey

The honey from *Trigona itama* stingless bees was obtained from the local kelulut honey breeder in Kuala Pilah. The bee-keeping activities and honey collections at this farm have been certified by MARDI in Malaysia. The certificate of analysis for the kelulut honey was performed by Hospital University Kebangsaan Malaysia (HUKM) and attached in Appendix D. The kelulut bees were multi-floral and reared using innovative green

technology, Technohive to preserve the natural habitat for the bees (a box that looks like a forest tree trunk and serves as a beehive for the kelulut bee). For the purpose of uniformity, the same batch of kelulut honey from *Trigona itama* was used in the study. As this was the first study with kelulut honey for wound healing in diabetics, sterilisation was not performed as the sterilisation protocol for kelulut honey has not been established. According to Ujilestari et al. (2023), Gamma irradiation is an effective method to ensure sterility of honey and enhance antibacterial properties. As honey seldom gets contaminated with microorganisms, the risk of contamination is likely to be low. Moreover, it was reported that Gamma radiation reduces moisture content and Vitamin C which is needed for wound healing. Moreover, previous studies conducted with raw honey as mentioned in the literature review did not impede wound healing in DFU. Hence, raw kelulut honey was used in the present study. Nevertheless, future investigations should explore on the sterilisation protocol for kelulut honey.

The collection of kelulut honey was performed with a sterile plastic pipette and transferred to a sterile 250 ml amber bottle before it is delivered to the orthopedic clinic where it is stored in a cabinet away from sunlight. Kelulut honey was labeled as H1. The illustration in Figure 9 shows the flow of the kelulut honey preparation for the study

Figure 9: Preparation of Kelulut Honey

3.9.2.2 Medical-Grade Manuka Honey Dressing

The medical-grade manuka honey dressing was purchased from the official distributor with the brand name Activon honey dressing. It contained 100% manuka honey produced in the United Kingdom where the raw honey was filtered and sterilised using Gamma irradiation. The honey came in a tube of 25 g labelled as H2 and was kept in a closed cabinet away from sunlight in the orthopaedic clinic. The medical-grade manuka honey packaged in tubes is shown in Figure 10.

Figure 10: Activon (Medical-grade Manuka Honey)

Kelulut honey and medical-grade manuka honey were placed in a sterile, amber bottle, labeled as 1 and 2, and kept in the treatment room. Before the application of kelulut and manuka honey in each respective group, all the DFUs were prepared by cleansing with normal saline aseptically. The application protocol for both the honey was similar where the required amount of honey was collected with a sterile pipette and evenly spread on the surface of the wound in each group accordingly. Honey dressing was changed every other day with aseptic dressing techniques. The size of ulcers was continuously measured on day 7 and day 14 in both groups. The protocol for honey application in Phase 2 is shown in Figure 11.

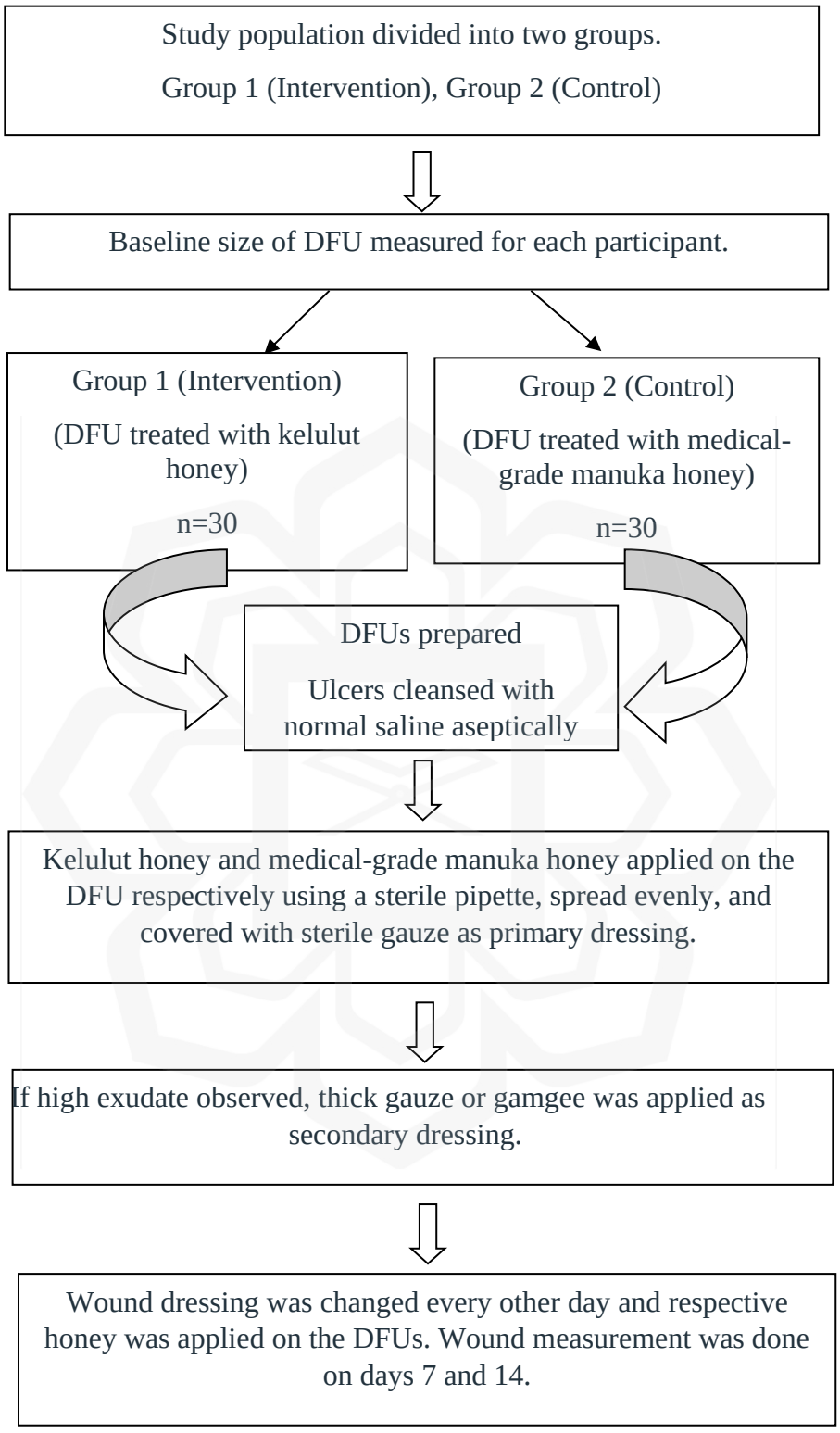

Figure 11: Phase 2 (Intervention Flowchart)

3.9.3 Treatment for Infection in DFU

Oral antibiotics were prescribed to the participants who had moderate infection based on the swab culture and sensitivity test report before the commencement of the study. Participants with severely infected wounds with resistant organisms were excluded from the present study. All the participants were given oral Unasyn 375 mg (1 tablet twice daily) as a standard infection control protocol in DFU and the medication was pursued for 14 days till the completion of the study. Unasyn 375mg contains 250 mg ampicillin/125 mg sulbactam per mL that has been recognised as one of the systemic antibiotics mainly used to treat moderate to severe infections related to soft-tissue infection (Olid et al., 2015) especially in DFU as per the IWGDF and IDSA guidelines (Senneville et al., 2023). The same type of antibiotics was prescribed to both groups accordingly to standardise the antibiotic administration. The participant would be removed from the study if infections worsened and swab test required admission for intravenous administration of antibiotics.

3.10 DATA COLLECTION PROCEDURE

The process of data collection was preceded by ethical approval and internal processing at UMMC with the approval for data collection and certificate for the credential and privileging. The principal investigator was headed by the foot and ankle orthopedic surgeon Dr. Nik Aizah Nabilla. The study team included the principal investigator, researcher, 2 medical officers, and 3 staff nurses. The study team was briefed on the study protocol with a focus on MDT and honey application by the researcher. To monitor the progress of the wound from baseline to days 7 and 14, the NDKare^{TM} mobile application software was uploaded to their respective handphones and it was password-protected. Data collection in the orthopedic outpatient clinic started in mid-December 2021. Participants were selected based on the inclusion and exclusion criteria using the consecutive sampling technique by the study team (researcher, principal investigator, and orthopaedic medical officers). All participants who agreed to be in the study were asked to sign the consent form. All baseline information on the 60 participants was recorded. The 60 consented participants underwent

debridement in Phase 1 with MDT was completed in a week. Then, they proceeded to Phase 2 which was the intervention phase with honey application. Before the intervention, the participants were divided into two groups (n=30 in each group) with a simple draw-lots technique with the kelulut honey intervention in Group 1 and Group 2 (medical-grade manuka honey). All information on the ulcer characteristics in both groups were recorded in the NDKare™ software. All ulcers were photographed using the NDKare ™ software in the study team's mobile phones and uploaded into the NDKare ™ database which could only be viewed by the principal investigator and researcher. The access to the database was also password protected. The ulcers were continuously photographed and uploaded on day 7 and day 14. Measurement of the ulcer was performed digitally by the NDKare™ software. It took 10 months to complete the data collection which ended in October 2022. Baseline data and ulcer size measurements from all 60 participants were compiled and analysed. The data collection process is summarised in Figure 12.

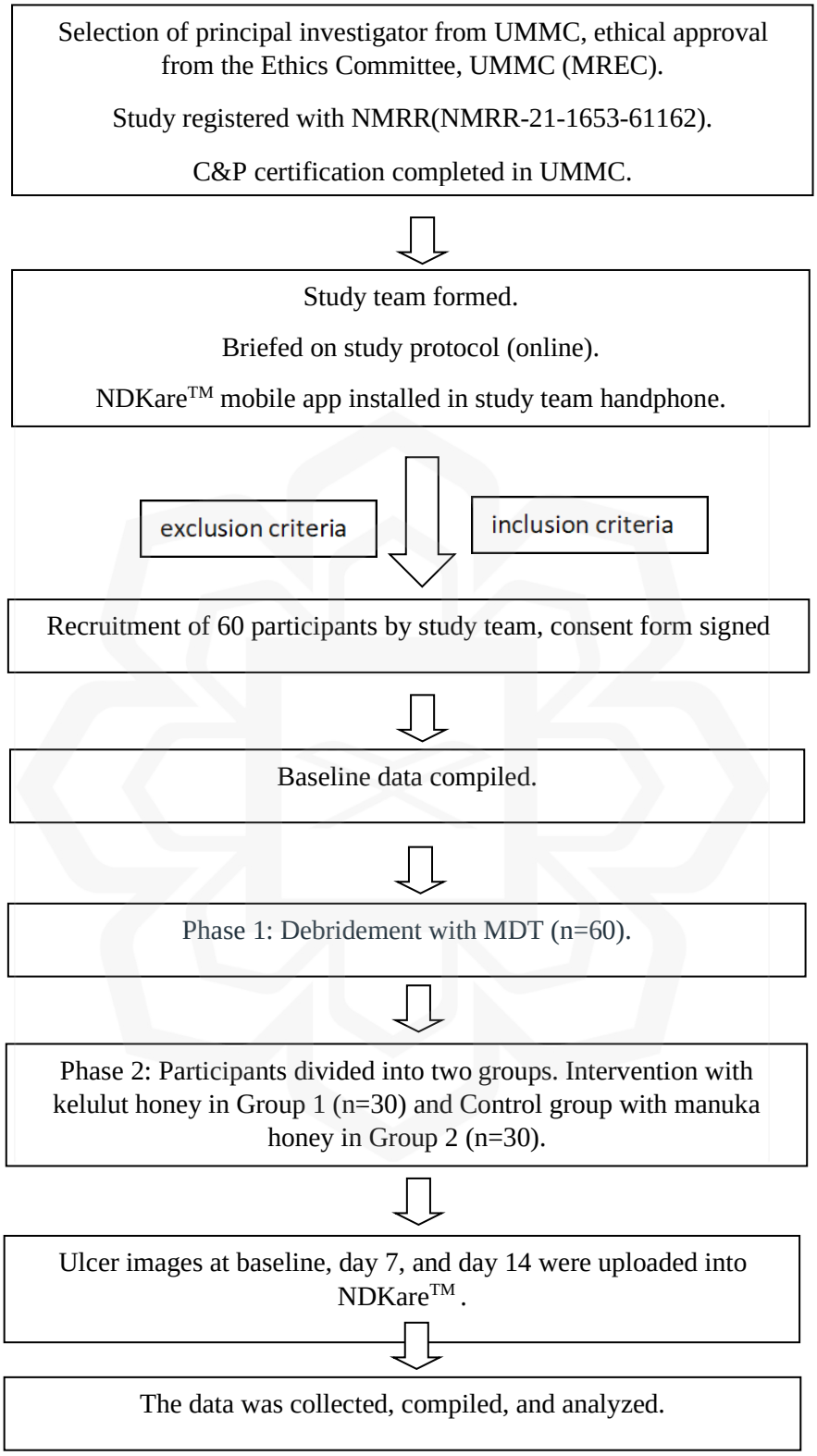

Figure 12: Data Collection Flowchart

3.11 ETHICAL CONSIDERATIONS

The ethical approval from the MREC of UMMC was done via an online application detailing a comprehensive proposal highlighting the objective of the study, study protocol, safety to patients, and the significance of the study. The application was spearheaded by the principal investigator from the Orthopedic Department. As part of the terms and conditions of receiving approval from the Ethics Committee of UMMC, the research was registered with the National Medical Research Register (NMRR) (NMRR-21-1653-61162). Approval for data collection at the outpatient orthopedic clinic in UMMC was preceded by internal processing which included an official data collection approval letter and Research Module certification for the Credentialing and Privileging (C&P) as attached in Appendix E and F. The MREC ethical approval letter is attached in Appendix G.

3.11.1 Informed Consent and Data Confidentiality

Informed consent is an agreement provided by the participants to the study team without any coercion or fear. The consent form was also prepared in English and Malay language and given to the participants according to their language preferences. The consent form was read and explained to participants who had reading difficulties by the researcher. The participants signed the consent form according to the language they preferred. The information on the research was communicated to all the participants before informed consent was sought. The information sheet included a detailed description of the research, either in Malay or English language (as preferred by the participants) was provided to the participants to alleviate any misunderstanding. Participants were given enough time to digest the information sheet before consenting to be enrolled in the study. The consent form and information sheet are attached in Appendix H and Appendix I, respectively. The participants were able to contact the researcher via the contact details provided in the information sheet. They were given the freedom to clarify any matters about the research before enrolment, during the ongoing study, and after the completion of the study. They were also informed that they could remove themselves from the study without any obligation to explain unless it was related to the study protocol. During the recruitment, participants' privacy was prioritised as they were enrolled in a private space in the

orthopedic treatment room. In the process of recruitment, the explanations were kept short, brief, and with an assurance that their data (socio-demography, clinical, and wound data) would be kept confidential. The participants were also informed that the outcome would be used in the thesis, and publications while the participants' details were kept undisclosed.

3.12 OUTCOME VARIABLE

The primary healing outcome of the present study was calculated based on the ulcer size reduction. The changes in the size of the ulcer were recorded after the intervention with honey, every week on day 7 and day 14. The secondary outcome was to assess the correlation between associated factors (age, gender, BMI, HBA1c, duration of diabetes, CKD, size, site, grade, duration of ulcer) and ulcer size reduction.

3.13 STATISTICAL ANALYSIS

Following the completion of the data collection process, statistical analysis was performed using the IBM Statistical Package for Social Sciences Version 29 to compare the efficacy of kelulut honey and medical-grade manuka honey in the treatment of DFU. The baseline characteristics of the participants and wound characteristics were differentiated into dependent and independent variables. Independent variables included the participants' socio-demography, clinical, and DFU characteristics at baseline whereas dependent variables consisted of ulcer size measurements on day 7 and day 14. Descriptive statistics were performed to assess if there was homogeneity in the baseline characteristics of the study population. Normality testing was performed to determine the distribution of data to select appropriate statistical testing to conclude if there exists any relationship between intervention and the outcome of the study. All the quantitative variables were checked for normality within each study group (kelulut honey and medical grade manuka honey groups). The categorical variables between the two groups were analysed using cross-tabulation and Chi-square test/Fisher's exact test. Normality in the distribution was tested

with skewness, kurtosis, and Shapiro-Wilk's test. A Shapiro-Wilk's test ($p>0.05$,), histograms, Q-Q plots, and box plots were inspected to determine the normality of data distribution in the study group and the size of ulcer at days 7 and 14. Normally distributed quantitative variables were compared by mean and standard deviation and independent sample t-test whereas non-normally distributed variables were tested using the Mann-Whitney U-test with the median and the inter quartile range. The differences between both groups were tested at a 95% confidence level and 5% significance level ($p<0.05$). For continuous data, Pearson correlation testing was performed to evaluate the relationship between age, HbA1c, duration of DFU, duration of diabetes mellitus, and size of the ulcer. Based on categorical data, Spearman's correlation testing was used to assess the relationship between gender, BMI, CKD, site, and grade of ulcer. The significance level of $p<0.05$ at 95% confidence was used to determine the statistical significance between the variables and, the outcome of the present study. To accept or reject the null hypothesis was based on the findings from the statistical analysis.

3.14 CHAPTER SUMMARY

This chapter described the methodology adopted for the present study focusing on the research design, protocol, ethical considerations, and statistical analysis. The methodology devised for the present study was based on previous studies with honey as mentioned in the literature review. A quasi-experimental study design was selected to evaluate the efficacy of kelulut honey and medical-grade manuka honey in the treatment of DFU. The outcome of the present study was based on ulcer size reduction from baseline to days 7 and 14. The instrument used to evaluate the outcome was NDKare^{TM} which was a mobile application software. The data was collected from 60 participants with DFU treated with kelulut honey in Group 1 and medical-grade honey in Group 2. Participants were ethically selected, informed about the aim of the present study, and included to participate. This chapter concluded with the statistical analysis methods to evaluate the collected data and fulfil the objectives of the present study. To the researcher's knowledge, this study is the first experimental research conducted on kelulut honey in DFU. Therefore, the research protocol

of this study may serve as a reference for further comprehensive and high-quality methodologies in future studies. The next chapter will present the results of the study.

CHAPTER FOUR

RESULTS

4.1 INTRODUCTION

This chapter reports the results obtained from the data analysis process to compare the efficacy of kelulut honey and medical-grade manuka honey in the treatment of DFU.

The segmentation of the chapter is shown below:

1. Description of the participants' socio-demography, clinical characteristics, and ulcer characteristics in the study population.

2. Normality testing to determine data distribution in Group 1 (kelulut honey group) and Group 2 (medical-grade manuka honey group).

3. To compare the efficacy of kelulut honey and medical-grade manuka honey in the treatment of DFUs based on the percentage of ulcer size reduction on day 7 and day 14 from baseline.

4. Assessment of the relationship between participants' socio-demography (age, gender, BMI), clinical characteristics (HbA1c, CKD, duration of diabetes), clinical characteristics of DFU (size, site, grade, duration of ulcer), and the percentage of ulcer size reduction.

4.2 DESCRIPTION OF STUDY POPULATION

The present study was conducted from December 2021 to October 2022 in UMMC. As mentioned in Chapter 3, a sample size of 60 adult diabetic participants with sloughy DFUs participated in this study. The participants were equally divided into two groups that received the kelulut honey (Group 1) and the medical-grade manuka honey (Group 2). Baseline characteristics, clinical characteristics, and ulcer characteristics of all 60 participants were recorded in Phase 1. The baseline measurement of ulcer size was done after Phase 1 and before the honey application in Phase 2. The baseline characteristics included age, gender, BMI, duration of diabetes, CKD, and HbA1c. The characteristics of DFU included grade, site, size, and duration of ulcer. The baseline characteristics for all the participants are summarised below in Table 14.

Table 14: Summary of Baseline Characteristics in the Study Population

Variable	Group 1 (Intervention) n=30	Group 2 (Control) n=30	Total n=60	*p-value*
Gender				0.184[b]
Male	(16)53.3%	(21)70.0%	(37)61.7%	
Female	(14) 46.7%	(9) 30.0%	(23)38.3%	
Age	64.5±11.20	65.4±12.27	65±11.65	0.768[a]
BMI				0.558[b]
Healthy (18.5-24.9)	(7)23.3%	(10)33.3%	(17)28.3%	
Overweight (25-29.9)	(16)53.3%	(12)40.0%	(28)46.7%	
Obese (30-39.9)	(7)23.3%	(8)26.7%	(15)25.0%	
HbA1c	8.13±1.80	8.08±1.32	8.11±1.56	0.909[a]
Duration of Diabetes	13.00±8.26	14.70±9.09	13.80±8.65	0.460[a]
CKD				0.190[b]
Stage 3	(15)50.0%	(10)33.3%	(25)41.7%	
Stage 1(normal)	(15)50.0%	(20)66.7%	(35)58.3%	
Grade of DFU				0.601[b]
Grade 2	(14)46.7%	(11)36.7%	(25)41.7%	
Grade 3	(16)53.3%	(19)63.3%	(35)58.3%	
Site of DFU				0.961[b]
Plantar	(8)26.7%	(6)20.0%	(14)23.3%	
Dorsum	(5)16.7%	(5)16.7%	(10)16.7%	
Forefoot	(7)23.3%	(7)23.3%	(14)23.3%	
Lateral	(5)16.7%	(7)23.3%	(12)20.0%	
Hindfoot/heel	(5)16.7%	(5)16.7%	(10)16.7%	
Duration of DFU (months)	2.40±0.99	2.40±1.04	2.40±1.01	0.801[a]
Size of DFU	33.64cm^2±30.80	28.21cm^2±28.8	31.0±29.71	<.001[a]

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[a] independent t-test, $p<0.05$ significant at 95% CI [b] Chi-square test, $p<0.05$ significant at 95% CI

4.3 DESCRIPTION OF BASELINE CHARACTERISTICS IN THE STUDY POPULATION

4.3.1 Gender

Gender was categorised into two groups (1=Male, 2= Female) and this categorical data were analysed to determine the homogeneity of gender between the two groups. Overall, the number of male participants was higher than female participants in the study population with 37 males (61.7%) and 23 females (8.3%). On average, the percentage of males in Group 1 was 53.3% and 70% in Group 2 while the percentage of females in Group 1 was 46.7% as compared to 30% in Group 2. Despite the differences in the gender profile, there was no statistical significance between the two groups (p=0.184). Therefore, males and females were homogenous between the groups.

4.3.2 Age

Based on the baseline data, the participants' age in the study population was between the range of 42 to 86 years old. The average age of participants in Group 1 was similar to Group 2 (65 years old). With the given mean age of 65 years old, it was estimated that half of the study population belonged to the age group below 65 years of age (between 42 – 65). Overall, there were no significant differences concerning age factors between the two groups (p=0.768).

4.3.3 BMI

BMI was calculated based on the participants' weight and height (kg/m^2). The overall BMI for majority of the study population fell into the overweight category (46.7%) followed by the healthy category (28.3%) and the least was in the obese category (25.0%). The BMI of participants under the overweight category was the highest in both groups (intervention 53.3% and control 40.0%). In Group 1, the BMI of participants under the obese and healthy were equally represented (23.3%) and in Group 2, the obese category was the least (26.7%).

However, there were no significant differences between both groups pertaining to the categories of BMI (p=0.558).

4.3.4 HbA1c

A hemoglobin A1C or HbA1c is a blood test that shows average glucose management over the past three months and used as an indicator for glucose control and a predictor for microvascular complications among diabetics (Cronin et al., 2017; Weykamp, 2013). Based on the National Glycohemoglobin Standardization Program in USA (NGSP), HbA1c is expressed as percentage of glucose bind to the hemoglobin in the red blood cells (Sherwani et al., 2016). Based on the baseline of HbA1c in the study population, the average measurement of HbA1c in Group 1 and Group 2 was almost similar (8%). The average reading of HbA1c of 8% indicated majority of participants had poor control of diabetes. Nevertheless, the HbA1c value in both groups showed no significant difference.

4.3.5 Duration of Diabetes

On average, majority of participants had 13 years of diabetes history in the study population. The duration of diabetes between Group 1 (13 years) and Group 2 (14.7 years). Despite the differences in the duration of diabetes, the analysis showed no statistical significance between both groups (p=0.460).

4.3.6 CKD

Overall, the percentage of participants having normal kidney function was higher than CKD stage 3 (58.3% and 41.7%) in the study population. The baseline data showed an equal representation of stage 1 and stage 3 in Group 1 whereas CKD stage 1 was higher than stage 3 in Group 2 (66.7% and 33.3% respectively). Despite the differences, the percentage of participants with CKD stage 1 and stage 3 was not statistically significant *(p=0.295*).

4.3.7 Grade of DFU

Participants with Wagner Grade 3 predominantly represented the study population in both groups with 53.5% in Group 1 and 63.3% in Group 2. Overall, the number of Wagner grade

2 was lower than grade 3. The differences in terms of Wagner grade of ulcer were not statistically significant (*p=0.601*).

4.3.8 Site of DFU

The site of the ulcer was categorised into dorsum, plantar, forefoot/toes, lateral, and hindfoot/heel. The data showed that there were more ulcers located in the plantar region of the foot (26.7%), forefoot/toes (23.3%), and equally located at dorsum, lateral, and hindfoot/heel (16.7%) in Group 1. In contrast, DFUs were more prominently located at the forefoot and lateral (23.3%) and the least at the plantar aspect (20.0%) in Group 2. There was an equal presentation of DFU at the dorsum (16.7%), forefoot (23.3%), and hindfoot/heel (16.7%) between the two groups. However, the differences in the percentage of ulcers at different sites between Group 1 and Group 2 were not statistically significant (*p*=0.961).

4.3.9 Duration of DFU

Based on the data, majority of the study population had a mean duration of ulceration of more than 2 months. However, the duration of DFU between Groups 1 and 2 was not significantly different at baseline (*p*=0.801).

4.3.10 Size of DFU

As shown in Table 13, the average size of ulcer in Group 1 was bigger as compared to Group 2 (33.64.0 cm^2 vs 28.21 cm^2). The analysis showed the average size of ulcer at baseline between both groups was not homogenous and significantly different between both groups (*p*<0.001).

4.4 NORMALITY TESTING FOR DATA DISTRIBUTION

The numerical values of skewness, kurtosis, and Shapiro-Wilk were used to determine the distribution of data in the intervention (Group 1) and control (Group 2) groups.

4.4.1 Data Distribution Based on Skewness, Kurtosis, and Shapiro-Wilk

Normality testing was performed using IBM SPSS Version 29. The values of skewness, kurtosis, and Shapiro-Wilk for the mean size of ulcer between Group 1 and Group 2 were checked. The results of the skewness, kurtosis, and Shapiro-Wilk showed the mean size of ulcers on day 7 and day 14 in both groups were not normally distributed. The results for skewness and kurtosis and Shapiro-Wilk are shown in Table 15 for Group 1 and Table 16 for Group 2.

Table 15: Data distribution in Group 1 (Intervention)

Group 1	Day	Mean size of ulcer, cm^2 (SD)	Skewness	Kurtosis	Shapiro-Wilk
Kelulut	Baseline	33.64(30.87)	1.406	1.509	<0.001
	Day 7	26.21(24.34)	1.470	1.738	<0.001
	Day 14	20.61(20.38)	1.478	1.717	<0.001

Table 16: Data Distribution in Group 2 (Control)

Group 2	Day	Mean size of ulcer, cm^2 (SD)	Skewness	Kurtosis	Shapiro-Wilk
Manuka	Baseline	28.21(28.77)	1.516	1.665	<0.001
	Day 7	27.34(27.39)	1.447	1.333	<0.001
	Day 14	26.17(26.18)	1.323	0.659	<0.001

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The descriptive statistics showed the average size of DFU for Group 1 and Group 2 at baseline was 33.64.0 cm^2±30.87 and 28.21 cm^2±28.77, respectively. On day 7, the average size of DFU was 26.21±24.34 in Group 1 and 27.34±27.39 in Group 2. On day 14, the average size of the ulcer was 20.61±20.38 in Group 1 and 26.17±26.18 in Group 2. The outcome for skewness, kurtosis, Shapiro-Wilk test ($p<0.05$) and visual inspection of their histograms, and normal QQ Plots showed that the mean size of DFU in both groups was not normally distributed. Therefore, there was enough evidence to reject the hypothesis that the variable follows a normal distribution.

4.5 COMPARING THE EFFICACY OF KELULUT HONEY AND MEDICAL-GRADE MANUKA HONEY IN THE TREATMENT OF DFU

As the distribution of data based on the mean size of DFU in the two groups was not normal and slightly skewed, non-parametric testing was found to be more appropriate to evaluate the efficacy of kelulut honey in the treatment of DFU. Thus, an independent sample Mann-Whitney U test was performed to compare the efficacy of kelulut honey (Group 1) and medical-grade manuka honey (Group 2) on day 7 and day 14 from the baseline. To compare

the efficacy of kelulut honey and medical-grade manuka honey, the percentage of ulcer size reduction was calculated based on the formula used in a honey-based study by Tsang et al. (2017).

The formula for the percentage of ulcer reduction:

ulcer size at baseline – ulcer size on day 7 / Ulcer size at baseline X 100%

4.5.1 Compare Percentage of Ulcer Size Reduction

Independent sample Mann-Whitney U test was used to compare the percentage of ulcer size reduction from baseline to days 7 and 14 with a significance level (usually α=0.05) between Group 1 and Group 2. Ranks were assigned to the values from the study population using the IBM SPSS Version 29. Independent sample Mann-Whitney U test statistic was generated based on mean ranks between both groups on day 7 and day 14. The outcomes of Mann-Whitney-U test statistics showing the mean ranks and sum ranks for the percentage of ulcer size reduction on days 7 and 14 between the two groups are illustrated in Table 17.

Table 17: Mann-Whitney U Test Showing Median, Mean Ranks, and Sum Ranks for Percentage of Ulcer Size Reduction Healing Between Groups on Days 7 and 14

Independent Sample Mann-Whitney U Ranks					
Test variable (Percentage ulcer size reduction)	Group	N	Median	Mean Rank	Sum of Ranks
Day7	Group 1 (Intervention with kelulut honey)	30	22.50	42.63	1279.00
	Group 2 (Control with medical-grade manuka honey	30	0.00	18.37	551.00
	Total	60	5.62		
Day14	Group 1 (Intervention with kelulut honey)	30	47.10	42.10	1263.00
	Group 2 (Control with medical-grade manuka honey)	30	7.50	18.90	567.00
	Total	60	16.67		

The results shown in Table 14 indicated that Group 1 (kelulut honey group) had a higher median percentage of ulcer size reduction (22.5%) with a mean rank of 42.63 on day 7 as compared to none in Group 2 (medical-grade manuka honey group). Similarly, the median percentage of ulcer size reduction in Group 1 was also higher (47.10%) with a mean

rank of 42.10 on day 14 as compared to the median percentage of ulcer size reduction (7.50%) with a mean rank of 18.90 in Group 2.

The test statistics for the Mann-Whitney U test between both groups on days 7 and 14 are depicted in Table 18.

Table 18: Mann-Whitney U Test Report Statistics Between Groups on Days 7 and 14

Independent Sample Mann-Whitney U Test Statistics[a]		
Test Statistics	**Percentage of ulcer size reduction on day 7**	**Percentage of ulcer size reduction on day 14**
Mann-Whitney U	86.000	102.000
Wilcoxon W	551.000	567.000
Z	-5.444	-5.152
Asymp. Sig. (2-tailed)	<.001	<.001

Table 15 above shows the actual significance value of the independent sample Mann-Whitney U test, specifically the test statistic, *U*, as well as the asymptotic significance (2-tailed) *p*-value. The data analysis yielded a significant difference in regards to the reduction in ulcer size (percentage) between both groups on days 7 and day 14 ($p<0.001$).

4.5.2 Effect Size Calculation for Mann-Whitney U Test

The IBM SPSS Version 29 did not provide an effect size statistic for the Mann-Whitney U test. However, the value of z that was reported in the output could be used to calculate an approximate value of the effect size.

$$r = Z/\sqrt{N}$$

Z = Z Statistics N = total number of cases.

1) Effect size, r calculation for percentage of size reduction on day 7.

   $= 5.444 /\sqrt{60} = 0.77$

2) Effect size, r calculation for healing rate on day 14.

   $= 5.152 /\sqrt{60} = 0.73$

According to Schäfer and Schwarz (2019), the effect size criteria are shown below:

0.1 = Small Effect 0.3 = Medium Effect 0.5 and higher = Large Effect

Based on the statistical findings, the effect size was 0.70 which indicated the difference in the percentage of ulcer size reduction had a large effect size (>0.5). Therefore, the efficacy of kelulut honey was approximated to produce larger effect in improving the healing outcome of DFU based on the percentage of ulcer size reduction on day 7 and 14.

Hypotheses

H_o: There are no differences in the efficacy of kelulut honey and medical-grade honey in the treatment of DFU on day 7 and day 14 from baseline.

H_1: There are differences in the efficacy of kelulut honey and medical-grade honey in the treatment of DFU on day 7 and day 14 from baseline.

Ulcer size reduction (percentage) on day 7

-median for intervention group=22.50, n=30, and median for control=0.00 (n=30)

(U = 86.00, z = 5.444, p < .001, r = 0.77)

Ulcer size reduction (percentage) on day 14

-median for intervention group=47.10, n=30, and median for control group=7.50 (n=30)

(U = 102.00 z = 5.152 p <.001 r = 0.73)

The statistical evaluation yielded a significant difference in regards to the ulcer size reduction (percentage) between both groups with a large effect on day 7 and day 14 from baseline. Overall, the percentage of ulcer size reduction was significantly higher with kelulut honey as compared to medical-grade manuka honey in the treatment of DFU on day 7 and day 14 ($p<0.001$). Therefore, the null hypothesis H_o was rejected.

4.5.3 Profile Plot Comparing the Percentage of Ulcer Size Reduction

A significant percentage of ulcer size reduction was achieved in the ulcers treated with kelulut honey as compared to ulcers treated with medical-grade manuka honey from the baseline to day 7 and day 14. A comparison in the percentage of ulcer size reduction between kelulut honey and medical-grade manuka honey is illustrated by the profile plot in Figure 13.

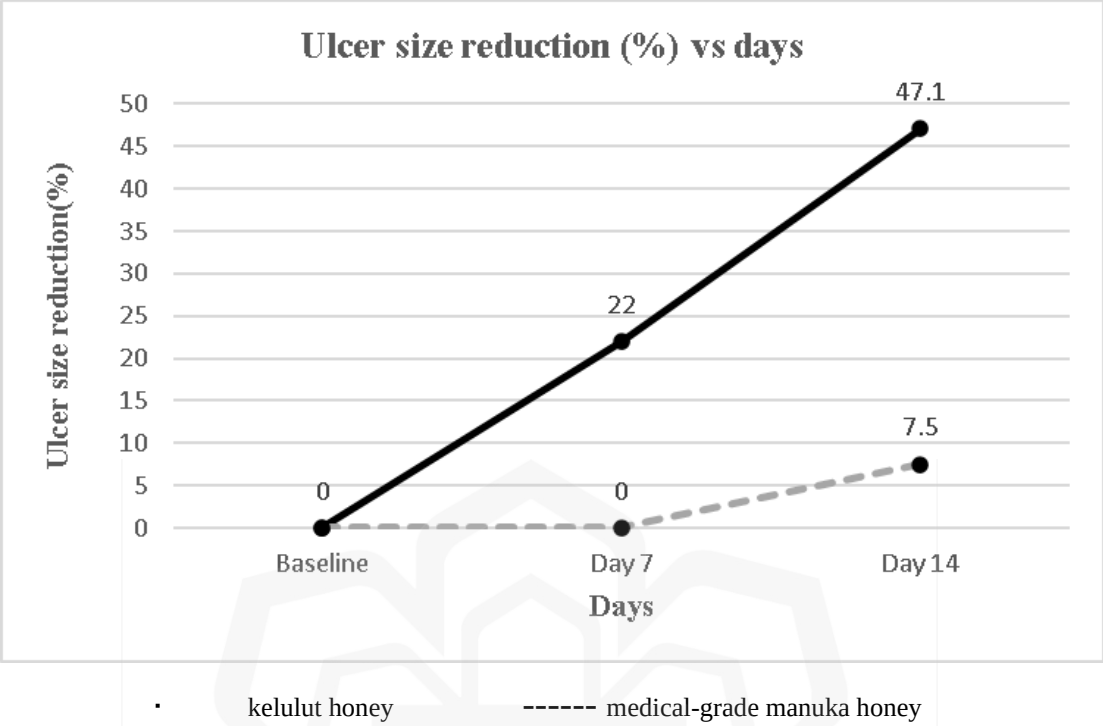
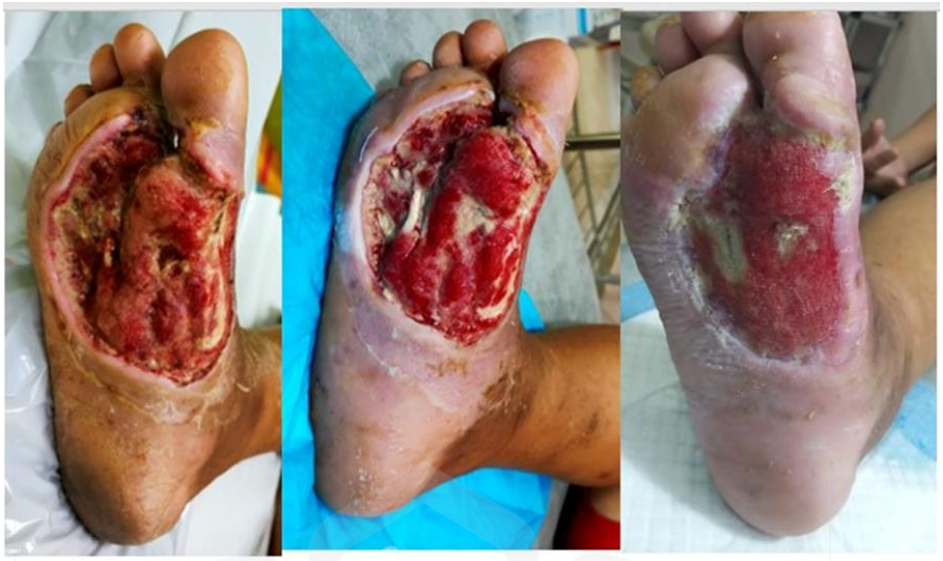


Figure 13: Profile Plot Comparing the Percentage of Ulcer Size Reduction Between Kelulut Honey and Medical-Grade Manuka Honey

4.5.4 Wound Images of Ulcers Treated with Kelulut Honey in Group 1 and Medical-Grade Manuka Honey in Group 2

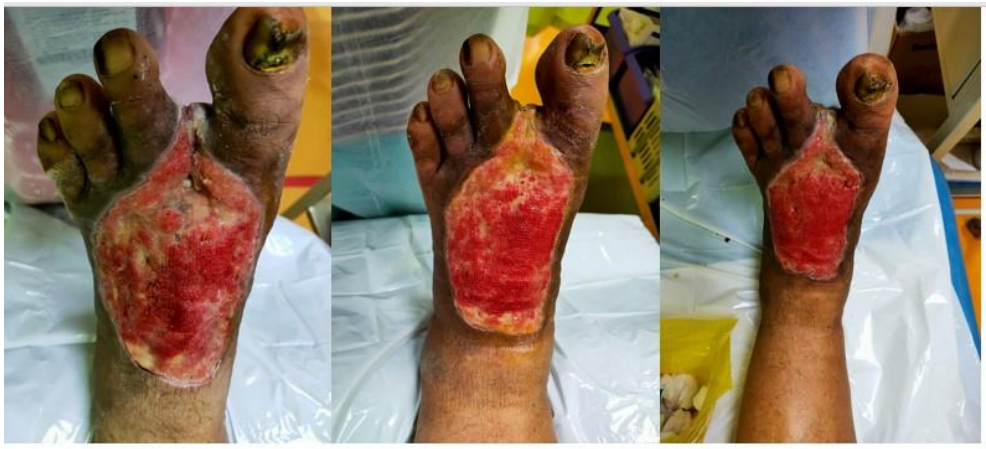
Some samples of wound images from Group 1 and Group 2 are attached for reference in Figures 14 and 15, respectively.



BASELINE DAY 7 DAY 14

BASELINE DAY 7 DAY 14

Figure 14: Changes in Ulcer Size in Group 1 (Kelulut Honey)



BASELINE DAY 7 DAY 14

BASELINE DAY 7 DAY 14

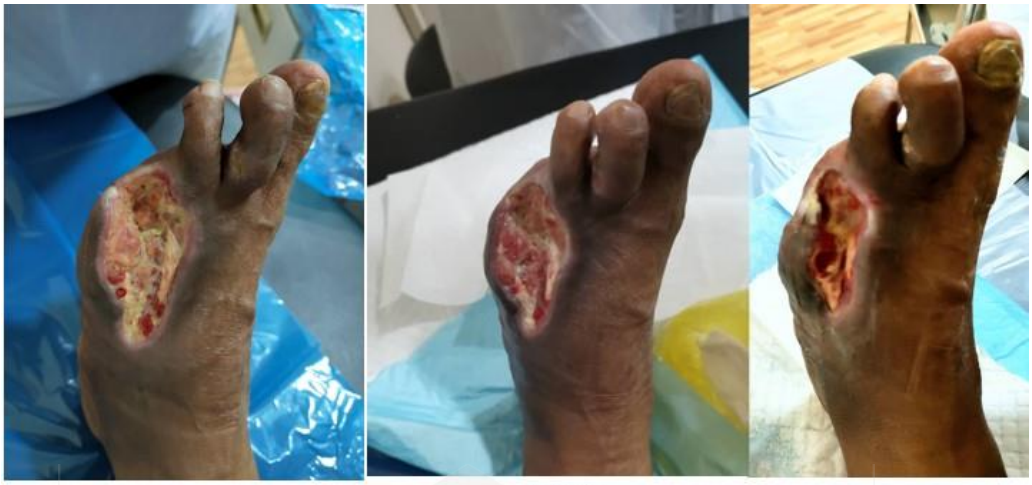


Figure 15: Changes in Ulcer Size in Group 2 (Medical-grade Manuka Honey)

4.6 EVALUATING THE RELATIONSHIP BETWEEN PARTICIPANTS' SOCIO-DEMOGRAPHY, CLINICAL CHARACTERISTICS, ULCER CHARACTERISTICS, AND ULCER SIZE REDUCTION (PERCENTAGE)

The second specific objective of this study was to determine the relationship between independent variables such as age, gender, BMI, CKD, HbA1c, duration of diabetes, site of ulcer, size, grade, duration of ulcer, and ulcer size reduction (percentage). The findings showed no significant correlation between the selected independent variables and ulcer size reduction (percentage). A summary of the statistical test results for the relationship between the independent variables and ulcer size reduction (percentage) is shown in Table 19.

Table 19: Relationship Between Independent Variables and Ulcer Size Reduction (percentage) (n=60)

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Variable	Statistical Test	Result	Conclusion
Age	Pearson Correlation	*r=0.13*	Positive, weak correlation
		p=0.334	Statistically not significant
Gender	Independent sample Mann-Whitney U test	*p=-0.537*	No significant association
BMI	Spearman Correlation	r_s*=0.081*	No significant association
		p=0.536	
CKD	Independent sample Mann-Whitney U test	*p=0.236*	No significant association
HbA1c	Pearson Correlation	*r= - 0.20*	Negative, weak correlation
		p=0.121	Statistically not significant
Duration of diabetes	Pearson Correlation	*r=0.085*	No correlation
		p=0.517	
Duration of ulcer	Pearson Correlation	*r=-0.027*	No correlation
		p=0.839	
Site	Spearman Correlation	r_s*=0.016*	No correlation
		p=0.905	
Grade	Independent sample Mann-Whitney U test	*p=0.356*	No association
Size	Pearson Correlation	*r=-0.012*	No correlation
		p=0.926	

*Correlation is significant, $p < 0.05$ at 95% CI

Based on the statistical findings shown in Table 18, Pearson's correlation was computed to investigate the direction and measure the association between age factor and percentage of ulcer size reduction in the study population of 60 participants. There was a positive, weak correlation between the two variables (r (58) =.13, p=0.334). This result indicated as the age increased, the percentage of ulcer size reduction also increased. However, the correlation between age and the percentage of ulcer size reduction was weak and not statistically significant.

As for variable Hba1c, the Pearson's correlation analysis result revealed a negative, weak correlation between HbA1c and healing outcome (r (58) =- .202, p=.121). The result indicated as the level of HbA1c increased, the percentage of ulcer size reduction decreased. However, the correlation between HbA1c and the percentage of ulcer size reduction was weak and not significantly correlated. Spearman's r*ho* indicated there was not enough evidence to show there was a correlation between the site of DFU and percentage reduction in the size of the ulcer based on the correlation coefficient. Similarly, with other statistical analyses, there was no notable significant association between gender, CKD, duration of diabetes, size, grade, duration of ulcer, and percentage ulcer size reduction in DFU among the 60 participants in this study.

4.7 OTHER OBSERVATIONS

Participants in the medical-grade manuka honey group (Group 2) complained of minor pain and uncomfortable feeling as soon as the honey was applied to the DFU. However, none of the participants had extreme pain during the point of study and needed analgesics. In contrast, no complaint of pain was documented among the participants in the intervention group with the kelulut honey (Group 1). As pain assessment was not included in the aim of the present study, pain score was not objectively evaluated and could be subjective observation reported by the participants in Group 2 treated with medical-grade manuka honey. Therefore, pain score can be measured in future studies to confirm the observation stated.

Another notable observation on the wound bed was maceration which was prominent in Group 2 whereas no maceration was observed surrounding the ulcer edges in Group 1 with kelulut honey. Maceration could enlarge the wounds and enhance skin breakage surrounding the wound edges. The maceration surrounding the edges of DFU treated with medical-grade manuka honey in Group 2 was prominently present during every dressing change as compared to Group 1. Consequently, the granulation process could be delayed in the healing of DFU with maceration (Tsuchiya et al., 2022). Overall, all participants did not require any surgical intervention or hospital admission for intravenous antibiotic administration during the data collection process in both Group 1 (kelulut honey) and Group 2 (manuka honey).

4.8 RESULT SUMMARY

The study population included 60 participants with an average age of 65 years old with the percentage of males was higher than females (61.7% and 38.3% respectively). The majority of participants had poor diabetes management as the average level of HbA1c was approximately 8.0 mmol in both groups. Most of the participants had a BMI of 25-29.9 which fell in the overweight category (46.7%) and 41.7% of them suffered from CKD stage 3. Grading of DFU using Wagner classification revealed majority of the participants had Wagner grade 3 than Wagner grade 2. The ulcers were predominantly located at the forefoot (23.3%) and the least at the heel and dorsum of the foot. The average history of DFU among the participants was recorded at 2.4 months which indicated delayed healing with conventional dressing on their own or at public health clinics or private clinics. The participants who were referred to the UMMC had chronic DFUs with different sizes ranging from 6 cm^2 to 90 cm^2 and with an average size of 31 cm^2. All the baseline characteristics such as age, gender, BMI, CKD, HbA1c, duration of diabetes, site of ulcer, grade, and duration of ulcer were homogenous between Group 1 and Group 2 except for the size of the ulcer. The size of ulcers in Group 1 treated with kelulut honey was averagely larger than Group 2 treated with medical-grade manuka honey (33.64 cm^2 vs. 28.21 cm^2).

Given the distribution of data was not normal and slightly skewed, a non-parametric test using independent sample Mann-Whitney U statistical testing was performed to evaluate the efficacy of kelulut honey compared to medical-grade manuka honey in the treatment of DFU on day 7 and day 14. The outcome showed the median percentage of ulcer size reduction in Group 1 (kelulut honey) was significantly higher than in Group 2 (medical-grade manuka honey) on day 7 (22.5% vs. 0%) and day 14 (47.10% vs. 7.5%). Independent sample Mann-Whitney U test revealed significant differences in the percentage of ulcer size reduction between both groups with a large effect size between both groups ($p<0.001$). Minor pain and discomfort were noted in Group 2 as compared to Group 1. However, no analgesics were prescribed for pain since none of the participants had an extremely painful condition. Maceration was also observed in Group 2 as compared to Group 1.

The result showed no association between the baseline socio-demography of participants, clinical characteristics, ulcer characteristics, and percentage of ulcer size reduction. However, age (and HbA1c showed an insignificant weak correlation with the percentage of ulcer size reduction that requires further investigations to confirm the association with the healing outcome in the present study. Overall, the outcome of the present study showed the percentage of ulcer size reduction was significantly higher with kelulut honey as compared to medical-grade manuka honey. Based on the findings, the application of kelulut honey in Group 1 showed a faster reduction of size on day 7 and day 14 as compared to no changes in Group 2 treated with medical-grade manuka honey on day 7 and changes in size were only observed on day 14. Statistical analysis demonstrated that the percentage of ulcer size reduction across time with kelulut honey was significantly higher as compared to medical-grade manuka honey in the treatment of DFU ($p<0.001$). The data analysis of the present study was verified by the Research Methodology and Statistical Consultation (RMSC) of UMMC as attached in Appendix J.

4.9 CHAPTER SUMMARY

This chapter presented the findings based on the statistical analyses performed to fulfil the two research questions. Given the non-normal data distribution between the two groups, non-parametric Mann-Whitney U testing was used to evaluate the differences between both groups based on the median ulcer size reduction (percentage). The results showed significant differences in the median percentage ulcer size reduction between both groups. Assessment of the relationship between associated factors in terms of the socio-demographic factors, clinical characteristics, ulcer characteristics, and percentage ulcer size reduction showed no correlation. The next chapter will discuss the findings of the study.

CHAPTER FIVE

DISCUSSION

5.1 INTRODUCTION

This chapter discusses the findings of the present study to evaluate the efficacy of kelulut honey as compared to medical-grade manuka honey post-maggot debridement therapy based on the percentage of ulcer size reduction at baseline, day 7, and day 14. It also discusses the relationship between the associated factors, including the participants' socio-demographic factors, clinical characteristics, ulcer characteristics, and ulcer size reduction.

5.2 COMPARING THE EFFICACY OF KELULUT HONEY AND MEDICAL-GRADE MANUKA HONEY BASED ON THE PERCENTAGE OF ULCER SIZE REDUCTION

Honey-based dressings have been used alternatively to accelerate healing in DFU due to their antibacterial, anti-inflammatory, and antioxidant properties, de-sloughing effect, and the ability to provide a moist wound healing environment (Naik et al., 2022; Smaropoulos & Cremers, 2019). The efficacy of honey has been evaluated based on various healing outcomes in the treatment of DFU such as healing rate, amputation rate, time to healing, length of hospital-stay, bacterial clearance, time to eradicate infection, ulcer size reduction rate, and complete wound closure (Purnama et al., 2021; Yildiz Karadeniz & Kaplan Serin, 2023). However, the size of ulcer is one of the commonly used variables by clinicians to monitor the progress of wounds and formulate treatment strategies for DFU. This is in line with the recommended guidelines set by IWGDF (2015) to prevent, monitor, and heal DFU. Based on the IWGDF guidelines, the size of ulcer was identified as one of the wound-healing predictors in the management of DFU (Forsythe et al., 2020; Game, 2016; Hinchliffe et al., 2020; Nicolaas Schaper et al., 2020).

According to Jeffcoate et al. (2018), reduction in the size of ulcer could lead to faster healing and reduce the risk of diabetes-based foot complications. It plays an important role in determining the healing trajectory of DFU (Patry et al., 2021). Several studies have highlighted the effect of ulcer size reduction on the healing of DFU. A reduction of 50% in the size of ulcer within 4 weeks is indicated as a wound healing predictor in 12 weeks. Larger DFUs > 5 cm^2 reportedly took longer time to heal in 24 weeks compared to sizes that are less than 5 cm^2. Furthermore, it was shown that DFU that failed to improve or worsen in 2 weeks or more had slower healing rates at week 12. According to Pickwell et al. (2015), failure to achieve a 90% wound size reduction at 8 weeks is a negative predictor for the healing of DFU at week 12. However, there were contradicting reports that mentioned a 50% size reduction at week 4 does not guarantee wound healing or closure at week 12n (Warriner et al., 2011). Despite the inconsistent outcomes, ulcer size reduction has been generally accepted as a healing predictor for DFU based on the IWGDF (2015). The percentage of ulcer size reduction has been highlighted in several studies as one of the healing outcomes measured in the management of DFU (Fife et al., 2016; Musa & Ahmed, 2012; Snyder et al., 2010; Tsuchiya et al., 2022). Therefore, the reduction of 47.10% in ulcer size with kelulut honey on day 14 as reported in the present study can be indicated as a wound healing predictor in 12 weeks.

Despite the size of ulcers in Group 1 being relatively larger at baseline (33.84 cm^2) compared to Group 2 (28.21 cm^2), the percentage of ulcer size reduction was higher in Group 1 compared to Group 2 on day 7 (22.0% vs baseline) and day 14 (47.10% vs 7.5%). It was not feasible to obtain similar sizes of ulcers in a short duration of time in an actual clinical setting for DFU management. A longer duration of data collection was anticipated if similar sizes of ulcers were to be included in the present study. Therefore, the researcher was unable to include participants with similar sizes of ulcers for both groups. Thus, no limitation was set for the size of the ulcer in the recruitment process due to time constraints. However, the selection of similar sizes of ulcers may reduce the risk of bias in determining healing outcomes associated with ulcer size reduction. Undoubtedly, it is more appropriate to obtain similar sizes of ulcers at baseline in clinical research. A longer duration of healing

toward closure may be required for bigger wounds despite achieving a higher percentage reduction in the size of the ulcer in the present study. Despite the differences in the ulcer size at baseline, the percentage of ulcer size reduction was significantly higher in Group 1 (treated with kelulut honey) than in Group 2 (treated with medical-grade manuka honey) on day 7 and day 14 as mentioned above ($p<0.001$). Faster ulcer size reduction within 7 days was achievable in ulcers treated with kelulut honey than in medical-grade manuka honey, which only showed a notable reduction in size on day 14 (7.5%). Therefore, kelulut honey was demonstrated to be more effective than medical-grade manuka honey in the treatment of DFU in this study. Given the statistically significant differences related to ulcer size reduction between both groups, the alternative hypothesis was accepted. However, the percentage of size reduction could not be associated with the healing rate of DFU in the two groups since the research protocol of the present study does not support the assessment of healing rate.

To the researcher's knowledge, the outcomes of this study could be the first to show a significantly shorter duration to achieve a higher percentage of ulcer size reduction on day 7 and further reduced on day 14 in DFU with kelulut honey (from *Trigona itama*). Undoubtedly, the findings concur with the previous comparison studies on honey and other types of dressing including conventional dressing in the treatment of DFU (Agarwal et al., 2015; Al Saeed, 2019; Chauhan et al., 2020; Imran et al., 2015; Jan et al., 2012; Kamaratos et al., 2014; Koujalagi et al., 2020; Tsang et al., 2017). Nevertheless, only two studies showed a direct comparison between honey based on the ulcer size. However, the results by Tsang et al. (2017) and Koujalagi et al. (2020) revealed a longer duration of healing as compared to the present study. The outcomes in the study by Koujalagi et al. (2020) are the most similar to the present study by achieving a significant percentage of ulcer size reduction of 49.87% on day 15 ($p<0.05$) with unprocessed honey compared to povidone iodine in the treatment of DFU. The outcomes demonstrated in this study are consistent with Koujalagi et al. (2020), which indicated honey as an effective wound dressing for the treatment of DFU. Given the similar sample size in both studies with 60 participants in the present study and 64 participants in the other, a solid conclusion may not be not drawn on the efficacy of honey in DFU. However, the finding in the present study could serve as a

preliminary outcome for kelulut honey using *Trigona itama* to achieve the shortest duration of healing based on the percentage reduction in the size of the ulcer.

Despite the similarities between the present study and Koujalagi et al. (2020), the accuracy of the measurement of ulcer size by the latter may be a matter of concern as a transparent graph paper was used to measure the size of ulcers as compared to validated digital wound application NDKare^{TM} in the present study. The transparent graph paper is a contact-measurement tool that may get stuck on the wound and create cross-contamination during the measurement as compared to the non-contact measurement tool used in the present study. Despite the significant ulcer size reduction outcomes on day 15, there was a lack of details on the participants' socio-demography, clinical characteristics, and ulcer characteristics that could influence the outcomes in the study by Koujalagi et al. (2020). Furthermore, the type of honey used in the study was also not mentioned whether it was from the honey bees or stingless bees. Despite being an RCT, the lack of information in the publication by Koujalagi et al. (2020) may affect the conclusiveness of the findings. Thus, the findings may not be objectively compared by the researcher to the present day.

The findings in the present study concurs with the healing outcome presented by Tsang et al. (2017) where honey is more effective than conventional methods (86.21% vs. 75.17%) in 12 weeks. Despite the positive healing outcome, the percentage ulcer size reduction in the present study with kelulut honey was higher as compared to Tsang et al. (2017). Moreover, the time taken to achieve a significant percentage ulcer size reduction was comparatively faster (7 days) in the present study. Overall, kelulut honey achieved a significantly higher percentage reduction in a shorter time despite the bigger ulcer size at baseline compared to Tsang et al. (2017) (33.82 cm^2 vs 10.98 cm^2). Nevertheless, the superior efficacy of nano-crystallised silver dressing over honey as reported by Tsang et al. (2017) could not be underestimated. As there is limited data available on the efficacy of silver-based dressing versus manuka honey dressing at this point, further investigation is required to evaluate the efficacy of kelulut honey against silver-based dressing. A direct comparison could not be made with several previous studies mentioned in the literature review as the research protocol of the present study was not designed to measure the healing outcomes in previous honey-related studies. For example, Agarwal et al. (2015) defined

healing outcomes as the duration of time taken to achieve readiness for surgical wound closure while Imran et al. (2015) evaluated complete healing as the research outcome. Similarly, wound healing score was evaluated by Karimi et al. (2019) whereas Jan et al. (2012) focused on the healing rate, Chauhan et al. (2020) focused on granulation and epithelisation rate, Kamaratos et al. (2014) and Al Saeed (2019) focused on honey's antibacterial effect and infection rate, while Bashir et al. (2018) compared the efficacy of negative pressure vacuum therapy and honey based on the readiness for skin grafting. Despite the contrast in the healing outcomes, the present study has strengthened the promising efficacy of honey in general and the clinical evidence of honey in improving wound healing outcomes for the treatment of DFU.

Despite the positive healing outcomes demonstrated by the previous studies, various limitations could have contributed to the differences in the findings compared to the present study. One of the main limitations is the ulcer measurement tool. As mentioned in Chapter 3, a validated software NDKare^{TM} application was used to measure the DFUs objectively in the present study at baseline, day 7, and day 14 compared to the conventional measurement tools used in the previous studies with honey such as the ruler technique (Imran et al., 2015; Kamaratos et al., 2014), transparent graph paper (Koujalagi et al., 2020), and measuring tape (Bashir et al., 2018). Nevertheless, it is commendable that two studies by Al Saeed (2019) and Tsang et al. (2017) used digital photography measurement that could validate their findings. Ruler and graph methods may not be appropriate for the measurement of DFU as they are considered invasive contact methods for the measurement of wounds which may invite cross-contamination (Shetty et al., 2012). Comparatively, digital photography is a more accurate and easier method. However, the limitation lies in the consistency of the distance of measurement whereby a fixed distance is required, and the imprecise measurement of wounds with cavities. Hence, underestimation in the size measurement is inevitable but digital wound measurement software is reported to be more accurate and non-invasive in the measurement of wounds (Chang et al., 2011).

The rationale behind the longer duration of wound healing in DFU than in the present study is the usage of different types of honey. Most previous studies used natural unspecified honey produced by honey bees *Apis sp*, except for Tsang et al. (2017),

Kamaratos et al. (2014), and Al Saeed (2019) who used manuka honey dressing produced by *Apis mellifera* like the present study. However, there were no comparison studies between kelulut honey and manuka honey or honey, in general, to evaluate if one is more effective than the other based on the percentage of ulcer size reduction in the treatment of DFU. Hence, more studies should be undertaken to establish the differences in the efficacy of honey produced by *Apis mellifera* (manuka honey) and *Trigona itama* (kelulut honey) in the healing of DFU. Another possible reason which could have contributed to faster healing time based on the ulcer size reduction in this study is the timing of the kelulut honey application. In clinical settings, honey is only considered when conventional dressing like saline or povidone dressing does not produce targeted healing outcomes in the treatment of DFU. Therefore, the inclusion of honey dressing as the first line of wound dressing as the soonest MDT was completed could have stimulated a faster wound healing outcome in the present study. Furthermore, the accessibility of both MDT and kelulut honey in Malaysia could be an added advantage in the explorations of this combination in future investigations.

Across the globe, honey is produced by honey bees and stingless bees, and mostly has similar therapeutic properties that could aid wound healing. Kelulut and manuka honey are known to have medicinal properties but they differ in their composition and potential benefits in promoting wound healing. As mentioned in previous studies, both the honey contained antioxidant capacity but they differed in their respective specific antioxidant compositions (Abu Bakar et al., 2017; Vyhlídalová et al., 2018). The composition of antioxidants in manuka honey is limited to flavonoids and phenolic acids, which have been indicated to be lower than in kelulut honey. As mentioned in Chapter 2, kelulut honey contains a range of antioxidants, including flavonoids, phenolic acids, and enzymes such as glucose oxidase (Imtiazah et al., 2021; Raja Nurfatin et al., 2021). Past studies have mentioned that kelulut honey contains potent phenolic acid, a type of organic compound that contributes to its antioxidant and anti-inflammatory properties. Moniruzzaman et al. (2014) mentioned the specific phenolic acids in kelulut honey were gallic acid, caffeic acid, and ellagic acid. Moreover, carotenoids found in kelulut honey such as beta-carotene, lutein, and zeaxanthin were shown to have anti-inflammatory and antioxidant properties. Other specific antioxidants found in kelulut honey include pinobanksin, pinocembrin,

quercetin, kaempferol, apigenin, and chrysin, which are all flavonoids with potent antioxidant activity (Abdullah et al., 2020; Labban, 2014; Meo et al., 2017).

The wound-healing effect of kelulut honey in the present study could have been made possible with its beneficial physiochemical therapeutic properties. A small number of reports have documented the beneficial effects of stingless bee honey in different contexts, such as antimicrobial, antioxidant, and anti-inflammatory activity. However, not all honey is the same and the physiochemical composition of antioxidants may differ based on different origins and species as highlighted in recent studies (Rao et al., 2016; Siavash et al., 2015). Considering kelulut honey's potent anti-oxidant properties (Selvaraju et al., 2019), the healing outcome with kelulut honey has been much anticipated. As clinical studies comparing the effect of kelulut honey and manuka honey in wound healing is lacking, in vitro studies related to differences in the antioxidant components for both types of honey can further rationalise the findings of this study. The rationale behind the positive healing outcome in the present study could have been attributed to the physiochemical properties of stingless bee kelulut honey as discussed in Chapter 2 and reported in previous studies (Kek et al., 2017, 2018; Maringgal et al., 2019; Mohamad et al., 2019; Omar et al., 2019). Given the link between the antioxidant phenolic compounds in the kelulut honey with its antibacterial and anti-inflammatory beneficial properties, positive wound healing outcomes in DFU are highly anticipated (Ávila et al., 2019; Carina Biluca et al., 2021; Ooi et al., 2021). The potency of antioxidants in kelulut honey is not solely dependent on the phenolic compounds, but also on flavonoids. Previous studies have clinically proven phenolic compounds and flavonoids correlated to reducing oxidative stress (produced by free radicals responsible for cell damage and death of cells), which has an indirect effect on reducing inflammation. The reduction of inflammation in wound healing is highly anticipated to improve healing outcomes in DFU and accelerate the healing process (De Almeida-Muradian et al., 2013; Duarte et al., 2012). Flavonoids have also been shown to stimulate cell proliferation and combat oxidative stress. Nevertheless, the number of antioxidants in kelulut honey or any other stingless bee honey may differ based on location, source of nectar, and species (Mustafa et al., 2018; Yaacob et al., 2017). Despite the absence of clinical data in wound healing with stingless bee kelulut honey, the antioxidant capacity may have largely contributed to the anti-inflammatory and antibacterial beneficial

efficacy in wound healing as demonstrated in several studies (Abd Jalil et al., 2017; Cianciosi et al., 2018; Meo et al., 2017). The potential of kelulut honey to eradicate infection with its low pH and high acidity is a great addition to wounds like DFU which face antimicrobial resistance (Mama et al., 2019; Nweze, Okafor, Nweze, & Nweze, 2017; Suntiparapop et al., 2012). The antibacterial properties of kelulut honey are further strengthened with the identification of the antibiofilm activity of this type of honey (Zamora et al., 2017). Biofilms have been a threat to DFU as these biofilms formed by bacteria are difficult to remove and slow down the healing process (Rahim et al., 2017).

Few other studies have supported the antibacterial effect of stingless bee honey in wound healing. Nevertheless, the body of evidence for kelulut honey has been further established with in vitro studies (Boorn et al., 2010; Omar et al., 2019; Ulasan et al., 2020).. In a recent study by Ranneh et al. (2018), the antioxidant in kelulut honey by *Trigona itama*was shown two times higher than in the honey bee *Apis dorsata* (locally known as Tualang honey). The findings concurred with earlier studies by Biluca et al. (2016) and Silva et al. (2013) which revealed the antioxidant potency of stingless bee honey. Despite the studies were not performed with *Apis mellifera* which is commonly found in Europe, the findings could be used as an indicator of the content of antioxidants present in honey bees as *Apis dorsata* is categorised under the same genus. A few reports have also emerged stating slightly higher antimicrobial activity of stingless bee honey as compared to manuka honey. In an in-vitro study by Ewnetu et al. (2013) in Ethiopia, the antibacterial effect of stingless bee honey and European-based honey bee was evaluated and the result showed both honey displayed different healing outcomes where stingless bees honey produced higher mean inhibition concentration against *Staphylococcus aureus*, *Staphylococcus aureus* (MRSA), *Klebsiella pneumonia*, and *Escherichia coli* as compared to honey bee *Apis mellifera*. Honey of the stingless bees produced a higher mean inhibition (22.27 ± 3.79 mm) compared to honey bee *Apis sp* (18.0 ± 2.3 mm). Commonly used antibiotics such as penicillin, ampicillin, erythromycin, methicillin, and amoxicillin were also used in the susceptibility test and the organisms were found to be resistant against these antibiotics. As these organisms are common in the infected DFU in clinical settings, the usage of stingless bee honey could be a potential alternative to treat resistant microorganisms in DFU. The antibacterial properties of stingless bee kelulut honey may have assisted in the

improvement of healing outcomes based on the percentage ulcer size reduction in this study.

In addition, certain enzymes in kelulut honey, such as glucose oxidase, catalase, and peroxidase, are also part of the body's antioxidant defense mechanism for the protection of cells from oxidative stress (Abdul Karim & Anum, 2019; Budin et al., 2017; Yazan et al., 2016). Moreover, the high flavonoid content in kelulut honey has been indicated to show potent free radical scavenging activity to defend cells against oxidative stress. Similarly, ascorbic acid in kelulut honey is a powerful antioxidant that aids in the protection of cells from oxidative damage. It is crucial to manage oxidative stress as it plays an important role in the pathogenesis of non-healing DFU. Oxidative stress is defined as the imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralise them with antioxidants (Dietrich et al., 2017; Giacco & Brownlee, 2010). In DFU, hyperglycemia and inflammation contribute to the high levels of ROS which cause damage to cells and tissue. Hence, the increase in ROS gives rise to oxidative stress which eventually delays the healing of wounds by disrupting tissue regeneration. Previous studies have shown that targeting oxidative stress with an anti-oxidant could provide scavenging of ROS, reduce damaging effects on cells and tissues, and improve wound healing outcomes in DFU (Dupont et al., 2020). A study by Yaacob et al., 2017) mentioned that oxidative stress could cause damage to deoxyribonucleic acid (DNA), proteins, and fats. Therefore, antioxidant properties in kelulut honey are a key factor in reducing oxidative damage, reducing inflammation, improving healing outcomes, and preventing foot complications among diabetics.

Despite the recognition received by honey bee *Apis sp* such as manuka honey compared to the species from a stingless bee, the total phenolic acid of stingless bee (*Plebeia spp*) was reported to be higher than *Apis sp.* which is 106.01 ± 9.85 mg GA equivalent/100 g compared to *Apis sp.* 92.34 ± 13.55 mg GA acid equivalent/100 g (Al-Hatamleh et al., 2020). Moreover, it was reported that total phenolic content was positively correlated with antioxidant activity in the stingless bee honey such as radical scavenging capacity, which is an indicator of positive healing outcome as demonstrated in the present

study (Duarte et al., 2012; Sousa et al., 2016). In the study by Ngaini et al. (2018), kelulut honey produced by the stingless bee *Trigona itama* in Borneo is a natural antioxidant that is four times higher than Tualang honey produced by *Apis dorsata*. Therefore, the antioxidant potency of kelulut honey compared to honey bee has been further strengthened by Ngaini et al. (2018) which may rationalise the outcome in the present study. The emerging in vitro evidence of kelulut honey is highly indicative of this type of honey to be explored for the treatment of DFU which is hard to heal with conventional methods. Further investigations could pave the way for kelulut honey to be considered as a potential wound dressing for the healing of DFU in preference to manuka honey.

Empirically, kelulut honey and manuka honey*)* do have similar therapeutic properties. However, recent reports have proven the superior potency of antioxidant compounds in kelulut honey, thus suggesting its potential as wound dressing for DFU (Al-Hatamleh et al., 2020; Nordin et al., 2020; Ramón-Sierra et al., 2020). Nevertheless, the potential of kelulut honey as wound dressing needs further investigations to confirm the findings in this study as location and species could make a difference in the therapeutic properties (Aina et al., 2018; Chuah et al., 2023). It can be summarised that kelulut honey has a higher potency of antioxidants which is a predominant compound for producing greater wound healing outcomes as compared to honey produced by *Apis sp* like manuka honey (Selvaraju et al., 2019; Zulpa et al., 2021).

5.3 RELATIONSHIP BETWEEN PARTICIPANTS' SOCIO-DEMOGRAPHY, CLINICAL CHARACTERISTICS, AND PERCENTAGE OF REDUCTION IN THE SIZE OF ULCER

The healing of DFU is a complex process that incorporates a variety of elements. Many publications have highlighted the association of patients' socio-demographic factors and clinical characteristics of the ulcers in the healing of DFU. However, factors related to the reduction in the size of ulcers with honey dressing are less common as limited explorations have been done on the efficacy of honey in the treatment of DFU. In this study, the

relationship between the participants' socio-demography (age, gender, BMI) clinical characteristics (duration of diabetes, CKD, HbA1c), and characteristics of DFU (size, site, grade, duration of ulcer) were assessed to fulfil the second objective. Age and HbA1c were revealed to be factors associated with the percentage of size reduction in the treatment of DFU.

5.3.1 Age

Diabetes and its complications become more common as people age, making DFU a common problem among the elderly. Based on the WHO (2020) report on Ageing and Health, "elderly" or "older person" was defined as any chronological age greater than or equal to 65 years. In Malaysia, the aging population has been on an upward trend, especially among diabetics, and the rate of wound healing tended to slow down when the ability of the skin to repair and remodel itself plunges (Everett & Mathioudakis, 2018; Makhtar, 2016; Tong et al., 2020). The healing of DFU among the elderly can be difficult due to a variety of aging-related issues. The influence of age on the healing of DFU has been extensively researched throughout the years. While the findings of different studies fluctuate, the general agreement is that older age is related to poorer DFU healing outcomes based on the consensus report in the IWGDF (Zhang et al., 2023).

It is generally understood that advancing age impairs the normal wound-healing process. Reduced tissue regeneration is one of the most difficult aspects of repairing DFUs in the elderly. The body's ability to make new tissue declines with age, which might hinder wound healing. Furthermore, they may have comorbidities that might impede healing, such as peripheral arterial disease (PAD), neuropathy, and renal impairment, all of which can affect blood flow, sensibility, and immune function, respectively (Liu et al., 2017). More than often, ageing has been indicated as a risk factor for delay in wound healing, especially among elderly diabetics and the growing aging population in the global context may increase the incidences of delayed wound healing. A few studies have indicated that age was an independent risk factor in the healing of DFUs with the elderly experiencing a slower rate of healing as compared to younger patients below 60 years of age (Chew et al., 2013; Ki et al., 2014). A recent article by Bonifant & Holloway (2019) also highlighted the

association between age and healing of DFUs based on the age-related changes in the layers of the epidermis and dermis that affect the skin's ability to resist injuries. It was mentioned that changes in the structure and volume of collagen in the dermis made the tissue stiffer and prolonged the closure of chronic wounds. Furthermore, occurrences of distal DFUs among diabetics are a matter of concern for the elderly as they struggle with mobility issues which make it challenging for them to adhere to wound care and treatment plans. Thus, elderly diabetics with DFUs tended to suffer from longer treatment duration and severe foot complications such as infection (Jia et al., 2017a). Moreover, elderly patients may have inadequate nutritional intake, altered hormonal responses, poor hydration, and compromised immune, circulatory, and respiratory systems, any of which can increase the risk of skin breakdown and impairment in wound healing (Thewjitcharoen et al., 2020; Zhao et al., 2016). Overall, age has been indicated as a risk factor for poor healing of DFU. However, studies exploring the associated factors influencing the reduction in the size of ulcer for DFU is under-researched.

However, the findings in this study showed otherwise where there was a positive but weak correlation between age and the percentage of ulcer size reduction in the healing of DFU. Nevertheless, the result was not statistically significant. Previous studies had shown inconsistent outcomes related to the association of age and healing outcome in DFU. The result conformed with the previous study by Al Saeed, (2019) given the study population is the middle-aged and elderly group belonging to the age group below 65 years of age (between 42 and 65 years old. However, the outcome in the present study contradicted Imran et al. (2015) which showed that age was not associated with the duration of healing outcomes in DFU. Given the inconsistent findings in the previous studies due to differences in the healing outcomes and lack of honey-related studies evaluating participant socio-demographic association based on ulcer size reduction, more investigations are needed to conclude the association. Categorisation of participants according to age for a longer period of observation could have yielded a different finding which was not able to be established in the present study. Furthermore, the timeline of observation was only 14 days which was a very short period to conclude the correlation. As mentioned earlier, the

relationship between age and healing outcome in the present study requires further investigation.

5.3.2 HbA1c

HbA1c, reflects glycemia throughout three months and has been used as the standard measure for the control of glucose among diabetics. There have been numerous studies evaluating the relationship between HbA1c and the healing rate. Majority of the findings demonstrated that elevated HbA1c was most associated with diabetic foot complications, poor healing of wounds, and lower limb amputations (Macdonald et al., 2018). A systematic review by Zeng et al. (2015) found that higher HbA1c levels based on NGSP were associated with a lower healing rate in DFUs, and suggested maintaining an HbA1c level below 7% could be beneficial in stimulating faster wound healing. Zhang et al. (2016) also highlighted a significant higher healing rate in patients with HbA1c < 7% compared to more than 7%. Recent studies also highlighted HbA1c > 8% as a risk factor for infection and lower limb amputations (Fesseha et al., 2018; Lane et al., 2020; Rastogi et al., 2022; Simoneau et al., 2021; Vas et al., 2020). Therefore, it is highly suggestive that poor control of diabetes with the increased value of HbA1c > 7% contributes to relatively higher incidences of diabetes foot complications. Given majority of the participants in the study had poor control of diabetes with an average HbA1c of 8%, it was highly anticipated that HbA1c to be negatively correlated with the healing outcome based on the percentage ulcer size reduction in the present study. The outcome of the present study corroborated with Al Saeed (2019) on the association between HbA1c and duration of wound healing that included higher mean of HbA1c > 9%. However, the findings of this study regarding HbA1c were not statistically significant as compared to Al Saeed (2019) which could be due to the differences in the mean value of HbA1c and shorter observation time in the present study (14 days vs. 2 years). However, there were inconsistencies in the inclusion of HbA1c in the previous studies mentioned in the literature review which included the exclusion of participants with HbA1c > 7% (Bashir et al., 2018; Imran et al., 2015), exclusion of participants with HbA1c > 10% but did not evaluate the effect of HbA1c on the healing of DFU (Tsang et al., 2017), and no mention of HbA1c in the study protocol (Agarwal et al., 2015; Chauhan et al., 2020; Jan et al., 2012; Kamaratos et al., 2014;

Koujalagi et al., 2020). Therefore, the result on the association of HbA1c and healing outcome with honey in DFU could not objectively cross-referenced. Therefore, future research on the efficacy of honey should consider the evaluation of HbA1c as a risk factor for the healing of DFU as numerous studies highlighted HbA1c to influence the healing rate of DFU in general (Al-Mohaithef et al., 2022; Almobarak et al., 2017; Pemayun et al., 2015; Schofield et al., 2021; Younis et al., 2018). In addition, fasting blood glucose monitoring could be taken into consideration as the base for diabetes management in the evaluation of DFU treatment as measurement of HbA1c alone may not be sufficient.

5.3.3 Gender, Duration of Diabetes, BMI, and CKD

Overall, there was no significant association between gender, duration of diabetes, BMI, and CKD indicated in the present study. The findings concurred with Al Saeed (2019) who concluded that gender was not related to the healing time of DFU as opposed to no evaluation of gender factors and healing outcomes in other honey-related previous studies as mentioned in the literature review. Despite having more males (61.7%) than females (38.3%) in the study population, there was no association between both genders in the healing outcome based on size reduction. Nevertheless, numerous studies related to wound healing in DFU in general have suggested inconsistent differences in the rate of healing between females and males. While Deng et al. (2022) showed no differences in the healing rate between males and females, number of studies indicated poorer wound healing among males as compared to females. Males were more prone to delayed healing in DFU as it was assumed they were highly engaged in outside activities compared to females and their plantar pressure is higher due to greater exposure hazards. Hormonal, genetic, and behavioral factors among elderly males were also mentioned to be the rationale behind the finding (Pedras et al., 2016; Vanherwegen et al., 2023). On the contrary, other studies have associated females to slower healing, higher amputation rates, and infection rates (Jia et al., 2017b; Musa & Ahmed, 2012; Ugwu et al., 2019). Therefore, the association between gender and wound healing in DFU remains inconclusive and still a matter of debate as some studies indicated females as a risk factor for DFU while others indicated otherwise in the healing of DFU as neuropathy, foot abnormalities, and poor footwear selection could affect both genders without bias. Hence, the association between gender and wound healing in

DFU remains inconclusive. Therefore, the gender aspect will need to be further evaluated when treating DFUs with honey. Concerning the present study, it is highly recommended that females and males be divided into different age categories to establish the association between gender and healing outcome in the treatment of DFU with honey.

BMI is a body fat measurement based on height and weight that applies to both adult men and women. It is a useful screening tool for identifying potential weight-related health issues but the impact of BMI as a risk factor for the healing of DFU has been less common. Despite the majority of participants in the study population belonged to the overweight category (25-29.9) and 25% of participants were in the obese category (30.0-39.9), the findings of this study showed that BMI was not associated with the percentage ulcer size reduction. Even though BMI was not mentioned in the previous studies with honey as a confounding factor in the treatment of DFU. Nevertheless, the outcome of the present study is consistent with several studies on DFU in general indicating no difference in healing time between overweight and non-overweight diabetics (Rosedi et al., 2022; Waaijman et al., 2014; C. Wang et al., 2019). However, there were shreds of evidence indicating a significant correlation between BMI and wound healing in the treatment of DFU in general. Previous reports suggested BMI in the obese category was associated with slower wound healing possibly due to poor circulation, compromised immunological function, and reduced tissue oxygenation (Al-Mohaithef et al., 2022; Eltilib, 2022). Obesity among diabetics also had been reported to lead to a higher risk for hyperlipidemia and vascular disease which could reduce blood supply to the lower limbs and delay wound healing (Chamberlain et al., 2022; Chen et al., 2023; McDermott et al., 2023; Rossboth et al., 2021). Moreover, achieving a healthy BMI through weight loss can be associated with improved wound-healing outcomes among diabetics and higher chances of wound closure. Therefore, the importance of BMI cannot be overlooked in accelerating healing and preventing foot complications in DFU (Sindhu, 2018). Given the number of participants in the obese category in the present study was only 15 (25%), the association between BMI and healing of DFU may require a bigger sample size and longer duration of observation to ascertain the relationship between both objectively. Further evaluations are

required to determine the optimal BMI range for the healing of DFU treated with honey based on the percentage ulcer size reduction as presented in the present study.

Several investigations have discovered that the duration of diabetes can have a considerable impact on the healing of DFU. In the present study, majority of participants had a history of diabetes for more than 13 years. However, the statistical analysis showed duration of diabetes was not associated with the percentage ulcer size reduction in the present study. Previous studies with honey in general did not evaluate the relationship between the duration of diabetes with the healing outcome of DFU except for Al Saeed (2019), which concurred with the present study. Nevertheless, the duration of diabetes was highlighted as a major predictor for delayed healing in DFU. The researchers discovered that patients who had prolonged diabetes take a longer time to recover from DFUs, and they concluded that early detection and care are crucial in preventing DFUs from becoming chronic (Deribe, 2014; Parisi et al., 2016; P. Zhang et al., 2016). A significant association between the duration of diabetes and the healing of DFUs was demonstrated by Skrepnek et al. (2017) whereby patients with a history of diabetes of more than 20 years had a 24% lower chance of wound healing than those who had diabetes for less than 10 years.

Prolonged exposure to high blood sugar levels also may cause blood vessel damage, making it more difficult for nutrients and oxygen to reach the wound site that could hinder wound healing. Similarly, medical problems such as neuropathy and vascular disease could also contribute to poor healing of DFU. Therefore, it can be rationalised that the longer a person has diabetes, they are prone to experience delayed wound healing with a higher risk for foot complications such as lower limb amputation. Age factor could also play a predominant role with the history of diabetes as diabetics of more than 10 years fall under the higher age group > 50 years old where the healing rate could be slower as compared to history of less than 10 years (Almobarak et al., 2017; Chiwanga & Njelekela, 2015; Ouyang et al., 2021). Despite the average age of participants (65 years) was higher among those living with diabetes for more than 10 years, the duration of diabetes was not associated with the healing outcome in the present study that did not corroborate with previous studies associated with the healing outcome of DFU in general. Therefore, the evaluation on the association between the duration of diabetes and healing of DFU may not be fully addressed

without the inclusion of a bigger sample size, age factor, and proper glycemic control (HbA1c < 7).

CKD is characterised by a deterioration in renal function among diabetics that develops into end-stage renal disease. Numerous studies on DFU have investigated the association between CKD and the healing outcome of DFU but none in previous studies related to honey-treated DFU as mentioned in the literature review. The occurrence of DFU and its poor progression appeared to be linked to the stage of CKD, with mortality peaking in individuals with end-stage renal disease (Jupiter et al., 2016). Healing of DFUs in patients with CKD is dependent on the stage of CKD and the presence of concomitant comorbidities (Dugbartey et al., 2022). In general, the earlier the stage of CKD, the greater the prospects of healing DFUs. People with early-stage CKD (stages 1 or 2) may have a fair prognosis for DFU healing, especially if they have good glycemic control and no substantial comorbidities. People with advanced CKD (stages 3, 4, or 5) may have a diminished ability to mend DFUs, especially if they have concomitant comorbidities such as peripheral artery disease or neuropathy (Skolnik & Style, 2021). Poor healing of DFU has been associated with the pathophysiology of CKD, which includes diminished blood flow, neuropathy, poor immunological function, and increased susceptibility to infections. About 40% of diabetics are anticipated to develop CKD, and 19% to 34% will experience DFU during their lifespan. Aljarrah et al. (2019) discovered that the stage of renal function was associated with healing prognosis and amputation risk. Grigoropoulou et al. (2017) also reported that individuals with CKD took a longer time to heal with a higher risk of amputation than those without CKD. Similarly, individuals with CKD were reported to have a significantly poorer healing rate than those without CKD. The incidences of non-healing DFUs were also observed to be considerably higher in individuals with CKD than those without CKD as reported by Lopez-de-Andres et al. (2015). Despite a small cohort of type 1 diabetics, Otte et al. (2015) established a negative connection between renal function and DFU, amputation incidence. In contrast, Chen et al. (2023) revealed that diabetes complications such as neuropathy and peripheral arterial disease were more significant predominant risk factors of poor healing outcomes than CKD stages. The findings of this are inconsistent with previous studies on DFU which highlighted the association between CKD and healing outcome of DFU, despite

the majority being in CKD stage 3. This could be because previous observations on DFU were done for a longer duration, with a minimum of a year (Bonnet & Sultan, 2022; Dugbartey et al., 2022) as compared to the present study. There is still inconsistent, insignificant, and lack of strong data addressing the relationship between CKD and the healing of DFU. Thus, a more comprehensive study design incorporating different categories of stages in CKD with different age groups and a longer duration of study may ascertain if CKD significantly influenced the healing outcome based on the size reduction with the intervention of honey in the treatment of DFU.

5.3.4 Size, Site, Grade, and Duration of Ulcer

Numerous studies have evaluated the characteristics of ulcer which includes size, site, grade, and duration of ulcer with the healing of DFU. In general, severity based on the grade of ulcer, site, size, and duration of ulcer had been shown to influence the healing outcome of DFU but certainly less in numbers for consistent findings (Abd Wahab et al., 2015; Kavitha et al., 2014; Smith-Strøm et al., 2017; Yasin et al., 2018). It was demonstrated there was a significant difference in healing rate between larger DFUs (more than 5 cm^2) and smaller ones (less than 5 cm^2) whereby larger DFUs took longer time to heal in 24 weeks. Similar findings were demonstrated in the latest study by Vahwere et al. (2023) with the healing outcome evaluated in 12 weeks. Another study discovered that larger ulcers greater than 2 cm^2 have a decreased chance of healing when compared to smaller ulcers. Overall, larger wounds have been consistently related to lower healing rates which may extend the healing time (Game et al., 2016). However, significant prospective studies with larger sample sizes are certainly limited to evaluating the predictors of DFU healing at present.

In the present study, participants had a severe grade 3 ulcer with a mean size of 31 cm^2 with majority of the ulcers located at the plantar and forefoot, and had the ulcers for an average of 2.4 months before seeking treatment in an outpatient setting at UMMC. Therefore, the condition of the DFU was presumably at a more advanced state. However, the statistical analysis showed there was no association between duration of ulcer and healing outcome in the present study which was not consistent with previous findings which indicated otherwise. In another study by Smith-Strøm et al. (2017), the duration of the ulcer

was revealed to be a predictor for the healing of DFU in 20 weeks of observation as compared to 2 weeks in the present study. The studies indicated the longer the ulcer existed, the longer it took to heal and demonstrated reduced healing rates**.** About the previous studies highlighted in the literature review, there were limited studies that explored ulcer characteristics and healing outcomes in ulcers treated with honey. Despite the similarities with the present study, the size and duration of the ulcer were significantly correlated with the duration of healing in the treatment of DFU (Al Saeed, 2019). To the researcher's best knowledge, similar significant relationship between size, duration of ulcer, and duration of healing was not shown in the last decade. A longer duration of the study with categorisations of size of ulcer based on 5 cm^2 as the benchmark and segmentation of duration of ulcer may be useful to assess the relationship between ulcer characteristics and wound healing with honey dressing in the future.

Similarly, categories in the site of ulcer were not associated with the healing outcome based on the percentage ulcer size reduction in the present study but there was a difference in the healing outcome between left and right foot as mentioned in the study by Koujalagi et al. (2020). The outcome in the present study corroborated with Tsang et al. (2017) whereby size, site, grade of ulcer, and duration of ulcer (12 weeks) were not correlated with the healing outcome of DFU. Offloading is also another important factor in determining the healing outcomes of DFU located in the plantar aspect. Therefore, the association of healing outcome based on the site of ulcer needs further evaluations to form a conclusion as there is limited knowledge on this subject matter. Similarly, the healing outcome of DFU could be affected by the duration of the ulcer as the longer an ulcer persists, the more difficult it is to treat and the slower it heals. Singh et al. (2017) mentioned that chronic ulcers such as DFUs that have been present for weeks, months, or even years are more likely to have massive tissue damage, infection, and other issues that might hamper recovery. Grade of ulcer also plays a vital role in determining the healing of DFU as higher grades of DFU tend to heal slower as compared to lower grades. For example, DFU with Wagner grade 4 tends to heal slower than grades 2 and 3 (Fife et al., 2016). Therefore, the duration of ulcer and grade of ulcer complemented with the site and size of ulcer need to be extensively studied to determine the association between ulcer

characteristics and the healing outcome of DFU. Overall, ulcers that are diagnosed and treated early could achieve a better healing rate and early treatment can help to keep the ulcer from worsening, reduce tissue damage, and promote faster healing. Furthermore, chances of healing could be optimised if prompt treatment is sought. while addressing the underlying conditions that contribute to ulcer formation can improve healing outcomes (Schmidt & Holmes, 2018).

5.4 CHAPTER SUMMARY

Both manuka honey from honey bee *Apis mellifera* and kelulut honey from stingless bee *Trigona itama* have potential benefits in enhancing the healing of DFU as demonstrated in the presented study. However, there were differences in the healing outcome for both the honey based on the percentage ulcer size reduction that indicated kelulut honey was more effective than medical-grade manuka honey in the treatment of DFU. The findings of this study indicated the percentage of ulcer size reduction in the kelulut honey group was significantly faster as compared to medical-grade manuka honey in the treatment of DFU on day 7 and day 14 ($p<0.001$). The percentage of ulcer size reduction on day 7 started with kelulut honey (22.5%) and continued to increase on day 14 (47.10%) whereas percentage of ulcer size reduction was only observed on day 14 (7.5%) in the medical-grade manuka honey group. EFF. Therefore, the Alternative hypothesis is accepted. Given the absence of scientific evidence comparing kelulut honey and medical-grade manuka honey in wound healing, the findings of this study may initiate more exploration into kelulut honey to improve wound healing outcomes in DFU. This proves kelulut honey as a potential wound dressing for DFU that can be optimised to reduce diabetes-based foot complications, which is a matter of concern of late. Therefore, the potential of kelulut honey as a wound dressing cannot be underestimated. Also, the finding has provided the much-awaited evidence for kelulut honey to be considered as a potential wound dressing in clinical settings. Despite previous findings indicating the therapeutic benefits of honey, the lack of robust evidence made it difficult for honey to be used in healthcare settings for wound management. Thus, the findings reported by this study have provided pivotal knowledge for kelulut honey to

be further explored to improve wound healing outcomes in DFU. The outcome of the present study also further strengthened the efficacy of honey in general for improving wound healing outcomes in DFU. However, the associated factors such as age, gender, BMI, HbA1c, CKD, duration of diabetes, size, site, grade, and duration of ulcer were not significantly correlated with the percentage ulcer size reduction in the present study. Hence, future investigations could explore these associated factors to determine the healing of DFU when treated with honey.

Based on the present study, kelulut honey from *Trigona itama* demonstrated a potentially superior shorter healing time based on ulcer size reduction in DFU compared to the previous studies related to honey in the literature review. To the researcher's best knowledge, the findings of this study could be the pioneer exploration indicating the potency of stingless bee honey in wound healing. Furthermore, the results demonstrated in the present study could establish and sustain the beekeeping economic-based activities for kelulut honey in Malaysia. Given the promising outcome with kelulut honey produced by *Trigona itama*, beekeeping activities of stingless bees or kelulut bees in Malaysia or any other countries must be expanded to widen the window of exploration. The outcomes displayed in this study may reduce the dependency on medical-grade manuka honey dressing and redirect the commercialisation of locally produced kelulut honey for wound management. Overall, kelulut honey from *Trigona itama* was indicated as an effective wound dressing for DFU as it produced a higher percentage of ulcer size reduction with a large effect size (> 0.5) as compared to honey from *Apis mellifera* in this study. Given the combination MDT-kelulut honey in this study, significant healing outcomes can be accelerated and pronounced to reduce the number of diabetes-based foot complications in healthcare settings. The next chapter shall conclude the outcomes presented in this chapter and provide recommendations for future studies.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 INTRODUCTION

The available body of evidence, as shown in the literature review, demonstrated the potential efficacy of honey in the treatment of DFU (Agarwal et al., 2015; Al Saeed, 2019; Bashir et al., 2018; Chauhan et al., 2020; Imran et al., 2015; Jan et al., 2012; Kamaratos et al., 2014; Karimi et al., 2019; Koujalagi et al., 2020; Tsang et al., 2017). The evaluation of kelulut honey in the present study is considered one of the first in the treatment of DFU since studies with stingless bee honey in wound healing are less common. The outcome conclusively showed that kelulut honey was more effective than medical-grade manuka honey dressing based on the percentage of ulcer size reduction on day 7 and day 14 from baseline ($p<0.001$). With kelulut honey, rapid reduction of wound size was achieved in 7 days and continued to reduce till day 14. The findings provided the much-needed body of evidence for kelulut honey as a potential wound dressing for the treatment of DFU. Expediting healing in chronic wounds such as DFU could overcome antibiotic resistance, and potentially prevent foot complications. The present study also revealed the independent associated factors related to socio-demographic factors, clinical characteristics and ulcer characteristics were not related to healing outcomes with honey treatment based on the percentage ulcer reduction. This chapter highlights the strength of the study, novelty, limitations, implications, recommendations for future studies, and ends with the conclusion of the study.

6.2 STRENGTH OF THE STUDY

The efficacy of honey in wound healing especially in the treatment of DFU is undoubtedly beneficial due to its antibacterial, anti-inflammatory, and anti-oxidant properties. Previous studies with honey, in general, have shown that honey is more effective than conventional dressing in the treatment of DFU. The present study highlighted the potential superior efficacy of kelulut honey by *Trigona itama* in the treatment of DFU based on the percentage ulcer size reduction compared to medical-grade manuka honey. The significant outcome revealed in the present study strengthened the efficacy of honey in general to enhance the healing of DFU which was not conclusively shown in previous studies as mentioned in the literature review. Overall, the efficacy of kelulut honey in the treatment of DFU has been demonstrated as a potential wound dressing to improve healing outcomes in DFU based on the percentage ulcer size reduction. Given the prominence of manuka honey as the preferred choice for honey dressing, the outcome of the present study is providing another choice for honey dressing based on kelulut honey. Therefore, further investigation with kelulut honey could reduce the dependency on the imported manuka honey dressing. To the researcher's best knowledge, the strength of the present study could be possibly attributed to the study design which had managed to achieve the objectives of the study.

Despite using the quasi-experimental study design, measures were taken to minimise rick of bias that included strict recruitment criteria, single blinding, simple randomisation technique, fulfilling adequate sample size of 30 participants in each group, and the selection of UMMC appropriate site selection. Reduction of bias, a crucial factor in the present study was an integral aspect that has been put into perspective throughout data collection. Despite the present study not being an RCT, comprehensive inclusion and exclusion criteria for the recruitment of participants and a simple convenient randomisation procedure were included. Undeniably, convenient/selective sampling was inevitable since it is not RCT. To reduce unnecessary risk of bias, the selection criteria including inclusion and exclusion criteria in the present study were comprehensively defined to reduce the effect of confounding factors in the healing outcomes. Participants with an ABI lesser than 0.8 were excluded from the study to rule out peripheral arterial disease (PAD) as ABI<0.8

is an indicator of PAD. The risk of higher incidences of infection in DFU is more prevalent in patients with PAD. Overall, the risk of bias was kept to the minimum especially during the recruitment of participants and throughout data collection with simple randomisation and single blinding in the present study. Another strength of the present study was no surgical intervention was involved in the treatment of DFU in the study population which could remove biases in the healing outcome of DFU. The usage of kelulut honey as a novel attempt to evaluate its efficacy in wound healing had established a significant base for further level 1 evidence investigations. In view of the promising outcome, the potential for kelulut honey in clinical settings could pave its way into the treatment protocol of DFU which may be an advantageous implementation in wound management.

In addition, the strength of this study was further enhanced with the novel combination of MDT and honey in the treatment of DFU. To date, no research has observed the combination effect of MDT and honey to improve healing outcomes as to the researcher's best knowledge. Based on the selective debridement effect of MDT, a well-vascularised stable wound producing a viable wound bed was optimised for kelulut honey to exert its therapeutic benefits to stimulate healing of DFU based on the percentage ulcer size reduction in the present study. The usage of a validated wound monitoring system is less common in majority of healthcare settings (Pena et al., 2020). The importance of proper, appropriate wound measurement tools for accurate and reliable measurement has been downplayed in the monitoring of wound progress and implementation of treatment strategies for chronic wounds. Pieces of evidence have shown that manual wound documentation may not be appropriate and efficient in monitoring wound progress and healing trajectory. Previously, majority of studies with honey measured the size of ulcers using graph paper, measuring tape, and ruler technique (Bashir et al., 2018; Imran et al., 2015; Kamaratos et al., 2014; Koujalagi et al., 2020) with fewer studies used digital photography techniques (Al Saeed, 2019; Tsang et al., 2017). However, recent pieces of evidence have shown that wound area measurements using conventional methods such as ruler, graph paper, and measuring tape were overestimated by 10% to 40%, less accurate, and imprecise (Bowling et al., 2013). In the present study, the usage of validated and accurate measurement application NDKare^{TM} app has strengthened the reliability of the

findings in the study. Furthermore, the NDKare™ app was given free of charge by the software's Malaysian agent and no cost was incurred in the wound documentation for the present study. The study conducted in Hospital Kuala Lumpur showed the usage of the NDKare™ application had saved 80% of the time over manual wound measurement, improved healing rates by 11%, provided accurate measurement of the length, width, perimeter, depth, and wound bed appearance such as granulation, slough, necrosis, bone with secured privacy of the patient (Nair, 2018). Several studies had also indicated better monitoring of DFU with the usage of non-contact mobile-based wound measurement application in healthcare settings (Amin et al., 2011; Shamloul et al., 2019; Yee et al., 2016). However, the usage of this software in wound documentation for research purposes remains less common in Malaysia. Therefore, it can be concluded that the wound documentation system using NDKare™ in the present study was the first to be utilised for honey-related studies in the treatment of DFU in Malaysia at the point of study. NDKare™ monitoring system remained user-friendly with no breakdown throughout the study and ensured the confidentiality of the participants' wound information was not compromised or data tampered throughout the study period.

The number of studies with honey in the treatment of DFU is still less in common despite the growing number of foot complications among diabetics with poorly controlled glucose. Given the limited comparison studies between kelulut honey and manuka honey in DFU, the outcome of the present study has established the potential of kelulut honey in wound healing. Studies have shown that the chronicity of DFU is time-dependent (Lobmann et al., 2020; Uccioli et al., 2015) and the result obtained in the present study highlighted faster healing time in DFU concurred. The strength of the study is attributed to the use of kelulut honey as the first line of treatment and not an alternative dressing. This much-needed evidence could lay a foundation for kelulut honey to be further explored as a potential wound dressing to improve healing outcomes in DFU. Overall, the present study has become a crucial point in the management of DFU because sufficient high-quality evidence are limited in the current publications concerning the efficacy of kelulut honey in DFU. Lastly, the uniformity of the present study was maintained till the completion of data collection in UMMC since only the researcher, principal investigator, and the study team

were involved in Phase 1 and Phase 2 of the study. No other medical personnel were involved in the application of maggot and honey, and documentation of data despite the challenges of quarantine issues during the data collection due to COVID-19. Moreover, the data analysis verification done by an independent party in UMMC (RMSC) further strengthened the findings with a reduction in bias factor.

6.3 LIMITATIONS OF THE STUDY

Even though the findings in the present study produced landmark positive wound healing outcomes for DFU with kelulut honey, there were a few limitations. Firstly, it's worth mentioning that the data collection for the present study was conducted during the outbreak of COVID-19 and UMMC was handling many COVID-19 admissions. As required, precautions were taken by wearing full PPE, masks, and gloves during data collection. The recruitment of participants was challenging during that point of time due to the strict standard operating procedure as physical contact during recruitment in Phase 1 and honey application in Phase 2 was limited. Quarantine aspect was also a limitation as the members in the study team including the researcher were quarantined periodically due to exposure to COVID-19 positive patients in the treatment clinic. In addition, the participants, researcher, and study team were tested practically every week for COVID-19 which was also a hassle for participants and many were not able to be included in the present study. Thus, the recruitment of participants took a longer time than anticipated. Despite keeping safe from COVID-19, the rigorous screening measures of COVID-19 made the data collection more challenging and time-consuming which was unavoidable.

There was also a limitation pertaining to the duration of observation (which was limited to 14 days) as it was a challenging period during the COVID-19 pandemic to recruit participants tested negative for COVID-19. A longer duration of follow up at least a minimum of 1 month could have been useful to evaluate the continuous efficacy of kelulut honey in the treatment of DFU. Despite the result showing a significant percentage size reduction in DFU treated with kelulut honey, it could not be conclusively correlated with

the healing rate as the follow-up duration was short, less than a month. A longer duration of follow-up also could have assisted in the evaluation of risk factors influencing the healing outcome in the study population. Inevitably, wound evaluations for 14 days were observed since a high drop-out of participants was anticipated during the pandemic. Amidst the challenges, data collection was successfully completed in 10 months. Furthermore, risk factor associations with the healing outcome of DFU could have been affected by the short duration of observation. The short observation period also could have affected statistical significance between HbA1c and the percentage of ulcer size reduction. Higher HbA1c levels indicating poor control of diabetes could have shown a significant correlation with the percentage of ulcer size reduction in the study if the duration of study was continued for 4 weeks or more. The outcome of association between diabetes management and healing outcome based on fasting blood glucose may have yielded a significant correlation with the percentage of ulcer size reduction. Given the fasting blood glucose was not measured during the data collection, the correlation between the diabetes management and healing of DFU with honey could not confirmed. As less exploration was conducted on the effect of Hba1c and fasting blood glucose in the treatment of DFU with honey, comparison could not be made objectively.

The limitation included small sample size despite being adequate in numbers for both groups. The inclusion of larger sample size could have been useful in determining the generalisation of the findings in the present study. With the limited sample size, the generalisability of the findings remains to be confirmed in future studies and the evaluation of the associated factors related to treatment of DFU with honey was not objectively feasible. The present study was also faced with limitations based on the non-similar size of ulcers at baseline as it was observed that recruiting large numbers of participants with identical etiologies such as similar size of DFU remained challenging in an actual healthcare setting. Different sizes of DFU were presented to the Orthopedic Clinic and due to the time constraint, the size of DFU was not considered in the inclusion criteria. Furthermore, in an actual clinical setting the majority of the DFUs have a wide range of sizes with the majority medium to large sizes as shown in the present study (the average size of DFU at baseline for the intervention group & control group was 33 cm^2 and about 28 cm^2 respectively). Thus, the non-homogenous size of DFU at baseline could serve as a

limitation to the present study. Given that the ulcers were only assessed physically, the microbiological assessment of infection throughout the data collection may have a confounding effect on the outcome of the present study that could be evaluated in future studies. In addition, the analysis of kelulut honey was not done prior to the study which could be a limitation for the study. However, the most recent analysis was performed by HUKM in 2018 as mentioned in the treatment protocol.

6.4 NOVELTY CONTRIBUTION OF THE STUDY

The management of chronic wounds such as DFU is facing a great challenge which results in significant patient morbidity and mortality. Furthermore, the nation is challenged with a huge healthcare burden in managing diabetes and its complications, especially foot complications (Doğruel et al., 2022). Moving back to the future could pave the way for an alternative effective treatment in improving wound healing outcomes.

There are three novelties contributed by the present study. Firstly, this is the first clinical study investigating the efficacy of stingless bee kelulut honey in the treatment of DFU. The majority of previous studies were conducted with honey produced by honey bees. Therefore, the positive outcome demonstrated in the present study highlights the potential of kelulut honey to be used as wound dressing for chronic wounds such as DFU. The promising outcome of kelulut honey shown could overcome the chronicity of DFU which has worsened due to antibiotic resistance in the past decade (Carter et al., 2016; Jain & Barman, 2017; Noor et al., 2017; Shaheen et al., 2022; Zubair, 2020).

Secondly, this is the first experimental study comparing two different types of honey in the treatment of DFU especially between *Apis mellifera* (honey produced by honey bees) and *Trigona itama* (honey produced by stingless bees). Much of the outcome in this study likely stemmed from the superior composition of antioxidants, which confers the anti-inflammation and antibacterial properties in kelulut honey as compared to MGO in manuka honey. As mentioned earlier, Malaysian kelulut honey produced by *Trigona spp* has been shown to have high total phenolic and total flavonoid content which are great

precursors to treat infection and enhance the healing of wounds (Abdul Karim & Anum, 2019; Mohamad et al., 2019; Ranneh et al., 2018; Yaacob et al., 2017). To the researcher's best knowledge, this is the first investigation that yielded a significantly faster ulcer size reduction with the application of honey in DFU.

Another novelty of the present study was the usage of MDT and kelulut honey combination for the treatment of DFU. To date, there have not been any publications on the evaluations of the combination of MDT and honey according to the researcher's best knowledge. The combination could trigger a double effect to expedite wound healing in DFU. Both MDT and honey have similar wound-healing properties whereby the capacity to overcome infection and stimulate healing is inadvertently present due to their antibacterial and antioxidant properties (Nair et al., 2020; Omar et al., 2019). In addition, the potency of MDT as an effective debridement tool could have improved the wound bed preparation and stimulated the healing process with the continuation of DFU treatment with honey to accelerate healing outcomes.

6.5 IMPLICATIONS

6.5.1 Clinical Practice

Several studies have highlighted the success of honey in eradicating resistant strains that conventionally failed to be treated in the management of DFU (Pleeging et al., 2020; Tashkandi, 2021). This could be one of the main factors why medical-grade manuka honey was considered as an alternative wound dressing for infected and biofilm-infested chronic wounds (Lu et al., 2019; Shirlaw et al., 2020). As the outcome of the present study showed superior healing outcomes with kelulut honey dressing than medical-grade manuka honey, the clinicians could consider using it to heal DFU to improve clinical outcomes. By managing antibiotic resistance, morbidity and mortality could be further reduced in the future if the wounds were to be stabilised in a shorter time. Hence, multi-drug-resistant pathogens and possibly biofilm which plays a huge role in delaying the healing of DFU could be removed (Martinotti et al., 2018; Yilmaz & Aygin, 2020). Therefore, kelulut

honey dressing could be a potent wound dressing to manage hard-to-heal and challenging DFU that has greatly impacted the lives of millions of diabetics across the globe.

6.5.2 Patient

The overall impact of non-healing DFUs has been quite devastating and reduced the quality of life for diabetics who mostly end up with foot complications such as infections and lower limb or digit amputation (Younis et al., 2018a). In addition to losing mobility, poor healing DFU created a great financial burden to the patient & family. The cost of treatment combined with prolonged treatment duration could be a physical and psychological trauma to the patient (González de la Torre et al., 2017).

Effective management of DFU to accelerate healing could provide the answer to alleviate the suffering of the patients. Conventional dressing was unsuccessful and ineffective in treating DFU due to various micro and macro complications as demonstrated in previous studies (Baltzis et al., 2014; Dietrich et al., 2017; McEwen et al., 2013). Hence, the utilisation of honey has been undeniably useful in improving wound healing outcomes in recent times (Lima et al., 2020). Based on the findings in the present study, the usage of kelulut honey could be beneficial for the treatment of DFU. Even though the cost-effectiveness of kelulut honey in wound healing has not been established yet, it can be forecasted that kelulut honey could be more cost-effective than medical-grade manuka honey since it is locally produced and the latter is imported. The accessibility of the locally-produced kelulut honey-based dressing may be more beneficial for patients suffering from chronic DFUs compared to imported honey dressings. Improving healing outcomes at a faster rate with kelulut honey could reduce the psychological trauma, pain, and immobility experienced by the patients. Acceleration of wound healing also could prevent foot complications and dependability on prosthetics in the event lower limb amputation is inevitable. Given the rising cost of medical treatment that includes prosthetics, prosthetics may not be accessible across the population (Andrews, Houdek, & Kiemele, 2015). Therefore, using kelulut-based honey dressing may provide an effective solution to

improve and expedite the healing of DFU that could play to the advantage of diabetics who suffer from poor healing wounds.

6.5.3 Bee-keepers

Based on a report by MARDI, 30 species of stingless bees (kelulut) have been identified in Malaysia. However, only 9 stingless bee species were highly domesticated due to their honey-producing capacity. It was reported that kelulut honey could reap about RM3 billion annually. The total market volume of the country's kelulut honey industry stood at 134,240 kg with sales of 19.3 million as reported by MARDI (Ismail & Ismail, 2018; MARDI, 2016). However, the total production of kelulut honey remained small compared to the market demand of more than 800,000 kg. In addition, the market potential stood at more than RM70 mil (Abdul Karim & Anum, 2019; Shamsudin et al., 2019; Mustafa et al., 2018). Therefore, the industry of kelulut honey has great potential as a viable source of income to improve the livelihood of beekeepers. The government via the 10-year plan could elevate the industry to become a potential economic revenue (Ismail & Ismail, 2018). As the demand increased, beekeeping activities in Malaysia have been enhanced in recent times. Undoubtedly, the economic contribution of kelulut honey could be stimulated further with the usage of kelulut honey for medical research such as wound treatment. Consequently, more income could be generated for the beekeepers and increased accessibility and availability of medical-grade kelulut honey for wound healing. The increase in beekeeping activities of kelulut honey may contribute to the country's economy indirectly and improve the livelihood of beekeepers. Despite honey produced by *Apis dorsata*, Tualang honey is a preferred choice for commercial purposes, risk of safety cannot be underestimated as painful stings from these bees were unavoidable and the location of hives could pose a risk for falls during honey collections. Therefore, beekeeping activities pertaining to stingless bees is safer and feasible.

6.5.4 Policy

The incidences of foot complications have been increasingly evident across the globe with 1 limb being amputated in 30 seconds as reported by WHO (2020) and IDF (2019). DFU has become a global threat to developing countries in terms of mortality and morbidity. In Malaysia, incidences of lower limb amputation prevalence have been rapidly increasing based on the demand for prosthetics. Given this fact, this upward trend posed a significant healthcare burden to the country. About RM1.4 billion was the estimated cost of the management of Type 2 diabetes and its complications in government hospitals (Lam et al., 2014). Since actual data for the prevalence of amputation was not available, it was acknowledged that the total expenditure on managing diabetes complications was humongous and possibly accounts for more than 50% of the total healthcare budget (Nair et al., 2022). It was estimated the annual direct outpatient cost of diabetes per patient was RM1,281 at community health clinics. In contrast, for public hospitals with or without specialists, the cost per admission in treating diabetes is nearly RM2,000. Although many local studies have explored the cost of T2DM previously, none have explored the actual total direct medical costs of diabetes complications (Arifin et al., 2017). Therefore, policies should focus on establishing audits for wound management including foot complications and provide avenues for research on this subject matter in order to search for alternative solutions with the aim to reduce the healthcare expenditure and prevent lower limb amputations.

Since the costs of managing foot complications remain high, the Ministry of Health Malaysia has a heavy task at hand to formulate effective policies. Based on the findings of the present study, policies should be drawn to enhance the production of kelulut honey, commercialise it as a wound dressing, and integrate it into the treatment of chronic wounds in public healthcare settings. Ultimately, effective policies to enhance the local economy using kelulut honey could benefit the country in the long run as stimulation of better treatment could prevent foot complications and improve productivity. The policies should be drawn to evaluate the quality of kelulut honey produced in Malaysia that could be similar to the UMF trademark for manuka honey set by the UMF Honey Association in New Zealand. Few studies have highlighted the importance of UMF rating for the quality

assurance of Leptospermum manuka honey in New Zealand (Girma et al., 2019; Johnston et al., 2018). The UMF is defined as a unique manuka factor that grades the quality of manuka honey-based mainly on the content of MGO, dihydroxyacetone (DHA), and leptosperine (a unique chemical marker for manuka honey). The UMF is considered a comprehensive quality assurance system that ensures natural properties, quality, and purity of manuka honey. The higher the UMF rating, the higher the antibacterial activity of MGO. Manuka honey requires a minimum of UMF 10+ to be therapeutically potent to improve overall health and overcome infections whereas UMF 25+ is more effective for chronic cases (Sherlock et al., 2010). New policies regarding the UMF rating can be drawn locally to ensure quality assurance of kelulut honey based on the antioxidant content and proceed for independent certification. Ultimately, the policy could improve the standards of kelulut honey and become the primary choice for consumption and wound healing.

6.5.5 Research

The beneficial effects of kelulut honey have been broadly shown in several local animal studies and lab-based studies in Malaysia highlighting the antioxidant& anti-inflammatory properties in kelulut honey (Mohamad et al., 2019; Omar et al., 2019; Ranneh et al., 2019; Ulasan et al., 2020). However, only one study explored the kelulut honey's capacity to increase human dermal fibroblast which could be useful for wound healing (Nordin et al., 2020). Therefore, more studies are needed to investigate the physiochemical properties of kelulut honey that could utilised to the advantage of wound care. The highlights of this research are clearly defined as the foundation for kelulut honey to be utilised for wound healing. Pointing to the given fact that clinical research on kelulut honey for wound healing remained limited with majority of research focused on manuka honey, the findings in the present study should widen the window of evaluations for kelulut honey in modern wound care. Further explorations into the cost-effectiveness of kelulut honey, antibacterial efficacy in antibiotic-resistant wounds, and action on biofilm are urgently required to overcome the ongoing battle in the treatment of chronically infected wounds. RCT with a bigger number of samples and longer duration using kelulut honey should be performed to produce Level

1 evidence to strengthen clinical evidence for kelulut honey in the healing of chronic wounds. A readily available locally produced medical product such as MDT and kelulut honey should be utilised to advance their usage as the prevalence of foot complications is on the rise. Research with longer duration of study and bigger sample size complying to comprehensive level 1 evidence should be conducted to advance the knowledge acquired from the present study.

6.6 RECOMMENDATIONS FOR FUTURE STUDIES

Despite the significant findings demonstrated by the kelulut honey in the treatment of DFU, it must be stated here that this study was only conducted on a small group of diabetic participants over a short period. Further research is needed to determine the long-term efficacy of kelulut honey with a much-standardised size of DFU in a larger group of participants before a generalised conclusion could be drawn. Given the significant wound healing outcome demonstrated in the present study, the window of exploration for stingless bee kelulut honey as a potential wound dressing should be expanded. To completely comprehend kelulut honey's therapeutic efficiency, more research is nonetheless required. The body of evidence presented in this study should initiate more research on kelulut honey's anti-bacterial activities on biofilm and other resistant microbial overcoming antibiotic resistance and improving healing outcomes in DFU. This is important to prevent the devastating impact of diabetes-based foot complications. Further investigations can compare the healing rate of kelulut honey and manuka honey in reducing amputation rate.

Upcoming studies should also include standardisations of honey in the study protocol as previous studies in the literature review included unprocessed, undiluted, sterile, and unspecified honey in the study protocol. The mention of whether the honey is sterilised or not and the species of honey should be clarified in the study protocol so that the findings on the efficacy of different types of honey can be confirmed. It is highly recommended that validated and reliable measurement tools such as NDKare^{TM} should be utilised to calculate the healing outcome based on ulcer size reduction rate. Inaccurate

usage of ruler and graph which are contact-methods for measurement of ulcers is suggested not to be considered to enhance the accuracy in future studies with kelulut honey. In addition, the study protocol could be further enhanced by expanding the location of data collection to more than one healthcare setting which may provide a better representation of the segmentation of the society. Kelulut honey from different locations in Malaysia could also be compared to evaluate the differences in the healing effect for DFU. The study protocol could be further sharpened to determine the wound healing trajectory of kelulut honey using RCT research design. RCT is highly recommended to produce level 1 evidence for honey to establish kelulut honey as a potential wound dressing in the treatment of DFU. Currently, several advanced dressings are being imported into the country for the management of wounds and burns. To the researcher's best knowledge, none of the advanced dressings which include negative pressure vacuum therapy, silver-based dressing, and oxygen therapy are produced locally. The majority of advanced dressings used in modern wound care are considered expensive (Lam et al., 2014; Mavrogenis et al., 2018; Naves, 2016; Rinkel et al., 2017; Wu et al., 2015). Therefore, it is crucial to evaluate the cost-effectiveness of kelulut honey compared to medical-grade honey to assess the applicability and feasibility of kelulut honey to be used as a potential wound dressing in healthcare settings. As managing DFU has become a costly affair for developing countries like Malaysia, exploration of the cost-effectiveness of kelulut honey could provide significant evidence for kelulut honey to be considered for DFU management. Given the medical-grade manuka honey dressing is currently being imported, the cost of purchasing manuka honey dressing is anticipated to be higher than locally produced kelulut honey-based dressing in the future. Therefore, the cost-evaluation of kelulut honey should be calculated taking into the consideration costs involved in the production of kelulut honey by the local beekeepers.

More experimental studies need to be conducted to evaluate the healing trajectory of DFU with the application of kelulut honey dressing with the combination of MDT. Clinical evidence on different types of chronic wounds such as venous ulcers could also be undertaken in future research as chronic wounds were not only confined to DFU (Elg & Hunt, 2018). When more level 1 evidence become available, commercial exploration in the form of gel and gauze-impregnated kelulut honey could be considered. Further

investigations could be carried out with different formulations in various healthcare facilities in Malaysia. The readily available sterile maggots of *Lucilia cuprina* and kelulut honey from stingless bee *Trigona itama* is an added advantage for future research to be performed locally as the present study would act as pioneer research for the combination of MDT-kelulut honey in the treatment of DFU. Lastly, future studies should consider including a control group in the study population which was a limitation in the present study. In addition, evaluation of the antibacterial aspect in kelulut honey can be studied further by including the evaluation of bacterial load based on microbiological-based laboratory testing which can be added as the outcome variable in future studies. Therefore, a larger sample size, longer duration of study, similar size of ulcer, cost-effectiveness, potency of antibacterial properties, pain score evaluations, comparative studies between different types of honey (*Apis sp* and *Trigona sp* or *Melliponi sp)*, standardisation of kelulut honey application are the factors to be considered in conducting future studies to produce level 1 evidence in terms of RCT to establish kelulut honey as an effective wound dressing for DFU.

Limited studies have explored on the effect of sterilisation on honey used in wound healing. Nevertheless, Gamma irradiation has been proven to reduce the microorganism activity in honey without affecting physical and some biochemical characteristics but it could reduce moisture content and Vitamin C in the honey. Few studies have indicated that Gamma radiation at 25kGy could reduce bacterial load, and enhance antioxidant properties of honey (Afifa Khatun et al., 2022; Hussein et al., 2011; Md Ibrahim Khalil et al., 2012; Molan & Allen, 1996). Bera et al. (2009) revealed there was no significant difference in the biochemical properties between sterilised and non-sterilised honey at 10 kGy Gamma radiation. In order to perform the sterilisation with Gamma radiation, specific dose need to be applied in order to sustain the therapeutic properties of the kelulut honey.To the knowledge of the researcher, there is a lack of information pertaining to the dose and the effect of Gamma radiation on the antibacterial propertie, physiochemical characteristics, antibacterial properties and antioxidant activity of kelulut honey at the point of time. Despite using raw kelulut honey, none of the wounds had severe infection during the data collection that is suggestive of low contamination risk. However, it is highly recommended

future evaluations on kelulut honey includes sterilisation with Gamma irradiation in order to produce medical-grade kelulut honey wound dressing.

6.7 CONCLUSION OF THE STUDY

The significant findings shown in the present study strengthen the efficacy of kelulut honey in wound healing since previous studies were not able to achieve significant outcomes. The present study is the first to demonstrate a potentially significant difference in the percentage of ulcer size reduction in the shortest duration with kelulut honey using *Trigona itama* as compared to medical-grade manuka honey of *Apis mellifera* in DFU ($p<0.001$). The higher percentage of size reduction with kelulut honey revealed that kelulut honey was more effective than medical-grade manuka honey dressing in the healing of DFU. Additionally, the present study also managed to bridge the gap between kelulut honey and wound healing outcomes in actual healthcare settings. Therefore, the role of kelulut honey in wound healing cannot be underestimated despite the availability of more popular manuka honey dressing in modern wound care. The associated factors influencing the healing outcome of DFU with kelulut honey which include age, gender, HbA1c, BMI, CKD, Hb, duration of diabetes, duration of ulcer, size, site, and grade of ulcer need further exploration as only age and HbA1c show a weak correlation due to the short duration of the study.

The outcome of this study has introduced new evidence for kelulut honey to be considered as a potential and effective wound dressing to improve the healing outcome of DFU based on the percentage of ulcer size reduction compared to medical-grade manuka honey. The outcomes also demonstrated that locally produced kelulut honey can improve the healing outcomes of DFU by significantly reducing the size of the ulcer at a faster rate on day 7 and continued to reduce the size till day 14 as compared to medical-grade manuka honey. Furthermore, the potential efficacy of kelulut honey in the treatment of DFU could assist clinicians in managing challenging and complicated chronic DFUs. Thus, the findings could expand the window of usage of kelulut honey from a nutritional base to a therapeutical angle in wound healing of DFU. However, more studies should be explored to investigate the therapeutic properties of kelulut honey to improve and expedite wound

healing in the management of DFU. Ultimately, patients' quality of life can be improved and the financial burden caused by costly treatments for wound management could be lowered and prevented. Hence, the beneficial therapeutic significance of using kelulut honey dressing should be taken into consideration in the management of DFU and reduce the dependability of imported medical-grade manuka honey dressing for wound treatment. Moreover, the novel combination of MDT-kelulut honey as shown in the present study could be integrated into the treatment protocol to further accelerate the healing rate of DFU.

In summary, the findings of the present study have yielded a significant healing outcome with kelulut honey based on the percentage of ulcer size reduction at a faster rate as compared to medical-grade manuka honey dressing in the treatment of DFU. As this is the first experimental study to demonstrate the efficacy of kelulut honey as a potential wound dressing in DFU, further evaluations are warranted to establish more clinical evidence for kelulut honey in wound healing. Thus, the potential of kelulut honey as wound dressing could be optimised to overcome antibiotic resistance and expedite the healing of DFU. Consequently, diabetes-based foot complications leading to severe infection and gangrene can be managed effectively to prevent lower limb amputations.

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APPENDICES

APPENDIX A1

EXPLANATION FOR THE JBI CRITICAL APPRAISAL TOOL FOR RCT

Explanation for the critical appraisal tool for RCTs with individual participants in parallel groups

JBI Manual for Evidence Synthesis. JBI, 2020.

Answers: Yes, No, Unclear or Not/Applicable

Critical Appraisal Tool for RCTs (individual participants in parallel groups)

1. Was true randomisation used for assignment of participants to treatment groups?

The differences between participants included in compared groups constitutes a threat to the internal validity of a study exploring causal relationships. If participants are not allocated to treatment and control groups by random assignment there is a risk that the allocation is influenced by the known characteristics of the participants and these differences between the groups may distort the comparability of the groups. A true random assignment of participants to the groups means that a procedure is used that allocates the participants to groups purely based on chance, not influenced by the known characteristics of the participants. Check the details about the randomisation procedure used for allocation of the participants to study groups. Was a true chance (random) procedure used? For example, was a list of random numbers used? Was a computer-generated list of random numbers used?

2. Was allocation to groups concealed?

If those allocating participants to the compared groups are aware of which group is next in the allocation process, that is, treatment or control, there is a risk that they may deliberately and purposefully intervene in the allocation of patients by preferentially allocating patients to the treatment group or to the control group and therefore this may distort the implementation of allocation process indicated by the randomisation and therefore the results of the study may be distorted. Concealment of allocation (allocation concealment) refers to procedures that prevent those allocating patients from knowing before allocation which treatment or control is next in the allocation process. Check the details about the procedure used for allocation concealment. Was an appropriate allocation concealment procedure used? For example, was central randomisation used? Were sequentially numbered, opaque, and sealed envelopes used? Were coded drug packs used?

3. Were treatment groups similar at the baseline?

The differences between participants included in compared groups constitute a threat to the internal validity of a study exploring causal relationships. If there are differences between participants included in compared groups there is a risk of selection bias. If

there are differences between participants included in the compared groups maybe the 'effect' cannot

be attributed to the potential 'cause' (the examined intervention or treatment), as maybe it is plausible that the 'effect' may be explained by the differences between participants, that is, by selection bias. Check the characteristics reported for participants. Are the participants from the compared groups similar with regards to the characteristics that may explain the effect even in the absence of the 'cause', for example, age, severity of the disease, stage of the disease, co-existing conditions and so on? Check the proportions of participants with specific relevant characteristics in the compared groups. Check the means of relevant measurements in the compared groups (pain scores; anxiety scores; etc.). *[Note: Do NOT only consider the P-value for the statistical testing of the differences between groups with regards to the baseline characteristics.]*

4. Were participants blind to treatment assignment?

If participants are aware of their allocation to the treatment group or to the control group there is the risk that they may behave differently and respond or react differently to the intervention of interest or to the control intervention respectively compared to the situations when they are not aware of treatment allocation and therefore the results of the study may be distorted. Blinding of participants is used to minimise this risk. Blinding of the participants refers to procedures that prevent participants from knowing which group they are allocated. If blinding of participants is used, participants are not aware if they are in the group receiving the treatment of interest or if they are in any other group receiving the control interventions. Check the details reported in the article about the blinding of participants with regards to treatment assignment. Was an appropriate blinding procedure used? For example, were identical capsules or syringes used? Were identical devices used? Be aware of different terms used, blinding is sometimes also called masking.

5. Were those delivering treatment blind to treatment assignment?

If those delivering treatment are aware of participants' allocation to the treatment group or to the control group there is the risk that they may behave differently with the participants from the treatment group and the participants from the control group, or that they may treat them differently, compared to the situations when they are not aware of treatment allocation and this may influence the implementation of the compared treatments and the results of the study may be distorted. Blinding of those delivering treatment is used to minimise this risk. Blinding of those delivering treatment refers to procedures that prevent those delivering treatment from knowing which group they are treating, that is those delivering treatment are not aware if they

are treating the group receiving the treatment of interest or if they are treating any other group receiving the control interventions. Check the details reported in the article about the blinding of those delivering treatment with regards to treatment assignment. Is there any information in the article about those delivering the treatment? Were those delivering the treatment unaware of the assignments of participants to the compared groups?

6. Were outcomes assessors blind to treatment assignment?

If those assessing the outcomes are aware of participants' allocation to the treatment group or to the control group there is the risk that they may behave differently with the participants from the treatment group and the participants from the control group compared to the situations when they are not aware of treatment allocation and therefore there is the risk that the measurement of the outcomes may be distorted and the results of the study may be distorted. Blinding of outcomes assessors is used in order to minimise this risk. Check the details reported in the article about the blinding of outcomes assessors with regards to treatment assignment. Is there any information in the article about outcomes assessors? Were those assessing the treatment's effects on outcomes unaware of the assignments of participants to the compared groups?

7. Were treatment groups treated identically other than the intervention of interest?

To attribute the 'effect' to the 'cause' (the treatment or intervention of interest), assuming that there is no selection bias, there should be no other difference between the groups in terms of treatment or care received, other than the manipulated 'cause' (the treatment or intervention controlled by the researchers). If there are other exposures or treatments occurring at the same time with the 'cause' (the treatment or intervention of interest), other than the 'cause', then potentially the 'effect' cannot be attributed to the examined 'cause' (the investigated treatment), as it is plausible that the 'effect' may be explained by other exposures or treatments occurring at the same time with the 'cause' (the treatment of interest). Check the reported exposures or interventions received by the compared groups. Are there other exposures or treatments occurring at the same time with the 'cause'? Is it plausible that the 'effect' may be explained by other exposures or treatments occurring at the same time with the 'cause'? Is it clear that there is no other difference between the groups in terms of treatment or care received, other than the treatment or intervention of interest?

8. Were outcomes measured in a reliable way?

Unreliability of outcome measurements is one threat that weakens the validity of inferences about the statistical relationship between the 'cause' and the 'effect'

estimated in a study exploring causal effects. Unreliability of outcome measurements is one of the different plausible explanations for errors of statistical inference with regards to the existence and the magnitude of the effect determined by the treatment ('cause'). Check the details about the reliability of measurement such as the number of raters, training of raters, the intra-rater reliability, and the inter-raters, reliability within the study (not as reported in external sources). This question is about the reliability of the measurement performed in the study. It is not about the validity of the measurement instruments/scales used in the study. *[Note: Two other important threats that weaken the validity of inferences about the statistical relationship between the 'cause' and the 'effect' are low statistical power and the violation of the assumptions of statistical tests. These other two threats are explored within Question 12).]*

9: Was follow-up complete and, if not, were differences between groups in terms of their follow-up adequately described and analyzed

For this question, follow-up refers to the period from the moment of randomisation to any point in which the groups are compared during the trial. This ques-tion asks whether there is complete knowledge (eg, measurements, observations) for the entire duration of the trial for all randomly allocated participants. If there is incomplete follow-up from all randomly allocated participants, this is known as post-assign- ment attrition. Because RCTs are not perfect, there is almost always post-assignment attrition, and the focus of this question is on the appropriate exploration of post-assignment attrition. If differences exist with regards to the post-assignment attrition between the compared groups of an RCT, then there is a threat to the internal validity of that study. This is because these differences may provide a plausible alternative explanation for the observed effect even in the absence of the cause (the treatment or intervention of interest). It is important to note that with regards to post-assignment attrition, it is not enough to know the number of participants and the proportions of participants with incomplete data; the rea- sons for loss to follow-up are essential in the analysis of risk of bias. Reviewers should check whether there were differences with regard to the loss to follow-up between the compared groups. If follow-up was incomplete (incomplete information on all participants), examine the reported details about the strategies used to address incomplete follow-up. This can include descriptions of loss to follow-up (eg, absolute numbers, proportions, reasons for loss to follow-up) and impact analyses (the analyses of the impact of loss to follow-up on results).

10. Was appropriate statistical analysis used?

Inappropriate statistical analysis may cause errors of statistical inference with regards to the existence and the magnitude of the effect determined by the treatment (‘cause’). Low statistical power and the violation of the assumptions of statistical tests are two important threats that weaken the validity of inferences about the statistical relationship between the ‘cause’ and the ‘effect’. Check the following aspects: if the assumptions of statistical tests were respected; if appropriate statistical power analysis was performed; if appropriate effect sizes were used; if appropriate statistical procedures or methods were used given the number and type of dependent and independent variables, the number of study groups, the nature of the relationship between the groups (independent or dependent groups), and the objectives of statistical analysis (association between variables; prediction; survival analysis etc.).

APPENDIX A2

EXPLANATION FOR THE CRITICAL APPRAISAL TOOL FOR QUASI-EXPERIMENTAL STUDIES (NON-RANDOMISED EXPERIMENTAL STUDIES)

Explanation for the critical appraisal tool for quasi-experimental studies (non-randomised experimental studies)

JBI Manual for Evidence Synthesis. JBI, 2020.

Critical Appraisal Tool for Quasi-Experimental Studies (Experimental Studies without random allocation)

Answers: Yes, No, Unclear or Not/Applicable

1. Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?

Ambiguity with regards to the temporal relationship of variables constitutes a threat to the internal validity of a study exploring causal relationships. The 'cause' (the independent variable, that is, the treatment or intervention of interest) should occur in time before the explored 'effect' (the dependent variable, which is the effect or outcome of interest). Check if it is clear which variable is manipulated as a potential cause. Check if it is clear which variable is measured as the effect of the potential cause. Is it clear that the 'cause' was manipulated before the occurrence of the 'effect'?

2. Were the participants included in any comparisons similar?

The differences between participants included in compared groups constitute a threat to the internal validity of a study exploring causal relationships. If there are differences between participants included in compared groups there is a risk of selection bias. If there are differences between participants included in the compared groups maybe the 'effect' cannot be attributed to the potential 'cause', as maybe it is plausible that the 'effect' may be explained by the differences between participants, that is, by selection bias. Check the characteristics reported for participants. Are the participants from the compared groups similar with regards to the characteristics that may explain the effect even in the absence of the 'cause', for example, age, severity of the disease, stage of the disease, co-existing conditions and so on? *[NOTE: In one single group pre-test/post-test studies where the patients are the same (the same one group) in any pre-post comparisons, the answer to this question should be 'yes.']*

3. Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?

To attribute the 'effect' to the 'cause' (the exposure or intervention of interest), assuming that there is no selection bias, there should be no other difference between the groups in terms of treatments or care received, other than the manipulated 'cause' (the intervention of interest). If there are other exposures or treatments occurring in the same time with the 'cause', other than the intervention of interest, then potentially the 'effect' cannot be attributed to the intervention of interest, as it is plausible that the 'effect' may be explained

by other exposures or treatments, other than the intervention of interest, occurring in the same time with the intervention of interest. Check the reported exposures or interventions received by the compared groups. Are there other exposures or treatments occurring in the same time with the intervention of interest? Is it plausible that the 'effect' may be explained by other exposures or treatments occurring in the same time with the intervention of interest?

4. Was there a control group?

Control groups offer the conditions to explore what would have happened with groups exposed to other different treatments, other than to the potential 'cause' (the intervention of interest). The comparison of the treated group (the group exposed to the examined 'cause', that is, the group receiving the intervention of interest) with such other groups strengthens the examination of the causal plausibility. The validity of causal inferences is strengthened in studies with at least one independent control group compared to studies without an independent control group. Check if there are independent, separate groups, used as control groups in the study. *[Note: The control group should be an independent, separate control group, not the pre-test group in a single group pre-test post-test design.]*

5. Were there multiple measurements of the outcome both pre and post the intervention/exposure?

To show that there is a change in the outcome (the 'effect') as a result of the intervention/treatment (the 'cause') it is necessary to compare the results of measurement before and after the intervention/treatment. If there is no measurement before the treatment and only measurement after the treatment is available it is not known if there is a change after the treatment compared to before the treatment. If multiple measurements are collected before the intervention/treatment is implemented then it is possible to explore the plausibility of alternative explanations other than the proposed 'cause' (the intervention of interest) for the observed 'effect', such as the naturally occurring changes in the absence of the 'cause', and changes of high (or low) scores towards less extreme values even in the absence of the 'cause' (sometimes called regression to the mean). If multiple measurements are collected after the intervention/treatment is implemented it is possible to explore the changes of the 'effect' in time in each group and to compare these changes across the groups. Check if measurements were collected before the intervention of interest was implemented. Were there multiple pre-test measurements? Check if measurements were collected after the intervention of interest was implemented. Were there multiple post-test measurements?

6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?

If there are differences with regards to the loss to follow up between the compared groups these differences represent a threat to the internal validity of a study exploring causal effects as these differences may provide a plausible alternative explanation for the observed 'effect' even in the absence of the 'cause' (the treatment or exposure of interest). Check if

there were differences with regards to the loss to follow up between the compared groups. If follow up was incomplete (that is, there is incomplete information on all participants), examine the reported details about the strategies used to address incomplete follow up, such as descriptions of loss to follow up (absolute numbers; proportions; reasons for loss to follow up; patterns of loss to follow up) and impact analyses (the analyses of the impact of loss to follow up on results). Was there a description of the incomplete follow up (number of participants and the specific reasons for loss to follow up)? If there are differences between groups with regards to the loss to follow up, was there an analysis of patterns of loss to follow up? If there are differences between the groups with regards to the loss to follow up, was there an analysis of the impact of the loss to follow up on the results?

7. Were the outcomes of participants included in any comparisons measured in the same way?

If the outcome (the 'effect') is not measured in the same way in the compared groups there is a threat to the internal validity of a study exploring a causal relationship as the differences in outcome measurements may be confused with an effect of the treatment or intervention of interest (the 'cause'). Check if the outcomes were measured in the same way. Same instrument or scale used? Same measurement timing? Same measurement procedures and instructions?

8. Were outcomes measured in a reliable way?

Unreliability of outcome measurements is one threat that weakens the validity of inferences about the statistical relationship between the 'cause' and the 'effect' estimated in a study exploring causal effects. Unreliability of outcome measurements is one of different plausible explanations for errors of statistical inference with regards to the existence and the magnitude of the effect determined by the treatment

('cause'). Check the details about the reliability of measurement such as the number of raters, training of raters, the intra-rater reliability, and the inter-rater reliability within the study (not to external sources). This question is about the reliability of the measurement performed in the study and it is not about the validity of the measurement instruments/scales used in the study. *[Note: Two other important threats that weaken the validity of inferences about the statistical relationship between the 'cause' and the 'effect' are low statistical power and the violation of the assumptions of statistical tests. These other threats are not explored within Question 8, these are explored within Question 9.]*

9. Was appropriate statistical analysis used?

Inappropriate statistical analysis may cause errors of statistical inference with regards to the existence and the magnitude of the effect determined by the treatment ('cause'). Low statistical power and the violation of the assumptions of statistical tests are two important threats that weakens the validity of inferences about the statistical relationship between the 'cause' and the 'effect'. Check the following aspects: if the assumptions of statistical tests were respected; if appropriate statistical power analysis was performed; if appropriate

effect sizes were used; if appropriate statistical procedures or methods were used given the number and type of dependent and independent variables, the number of study groups, the nature of the relationship between the groups (independent or dependent groups), and the objectives of statistical analysis (association between variables; prediction; survival analysis etc.).

APPENDIX B

INFORMATION ON NDKare app


NDKare™ -
Smart Wound
Care Solution
MEDICAL HISTORY
ALLERGIES
CURRENT MEDICATION
Key Benefits
Enhanced Patient Experience
Increased Productivity and Accuracy
Improved Care Outcome
Secured Online Collaboration
Realistic 3D Wound Model
It uses Advance Image Processing & Artificial Intelligence Technology to overcome challenges in the Wound Care Industry.
Quick & Easy Documentation
It enables Hassle-Free Wound Documentation in seconds - as easy as taking a photo!
Facilitate Collaboration
It connects patients, healthcare providers and medical supplies in just one sharing platform.
NDKare™ Medical Device is US FDA, Europe CE Mark and Singapore HSA registered.
FDA
HSA
Email us at
support@nucleusdynamics.com
Find more about us at
www.ndkare.com
Download NDKare™ Medical App
Download on the App Store
GET IT ON Google Play

Prospective Cohort Study to Validate Accuracy and Repeatability of Non-Contact Pressure Ulcer Wound Measurement

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HOME NURSING FOUNDATION 家护基金

BACKGROUND

In Singapore, the number of patients with chronic wounds is rapidly increasing and the financial burden on the healthcare system and family is significant. Chronic wounds not only causing suffering and worries to the patient, it has an impact on patients' families in regards of financial worries (Jabrink et. al., 2016). Wound dressing made up about 41% of the home visits conducted by Home Nursing Foundation.

Measurement is one of the important component in assessing wound healing. Linear measurement has been shown to predict wound healing that is independent of initial wound size or shape (Gorin et. Al., 1996; Gilman, 20014). Our nurses perform wound measurement by using a paper ruler (length and breadth) and swab stick (for depth) during their home visits which allows for assessing the progress of wound healing, as well as the effectiveness of the dressing therapy. To facilitate multidisciplinary discussion on the wound condition, the nurse would take a photograph of the wound. The quality of the photograph is dependent on lighting conditions, quality of the phone camera and distance from where the picture is being taken. Following this, the nurse would document the progress of the wound in our electronic medical record (EMR) system.

NDKare is a mobile apparatus that is able to accurately measure wounds and provide parameters such as length, width, perimeter and wound colour appearance. It also allows for seamless capturing, storing, uploading of digital photographs into our electronic medical record. An inter-rater reliability study of the device was undertaken by the developer of the solution. The coefficient for length, width, circumference and area lies within 1%, indicating a low level of variation between individual readings and hence, a repeatability of the same.

AIM

To compare and validate the accuracy of the non-contact wound measurement using a 2D and 3D digital photography against manual linear planimetry measurements for length, width and depth before adopting this solution for clinical utilisation.

METHODOLOGY

Study Design

The design of the study is a prospective, cohort study that was approved by HNF Ethics Committee. To avoid biasness, the measurements of length and width were measured in a double blinded procedure. This is accomplished by having investigators to obtain the manual planimetry measurement and digital photography. Manual measurement information and result of measurements of digital photography from eMedicareTM are tabulated and compared at the end of the study by an independent person who did not participated in any manual measurement or formulating the result of the digital photography. The intra-rater reliability of wound depth measurements taken by NDKare was also validated by recording 2 videos of the same wound. A total of 5 Nurse Clinicians were trained in 2 sessions to ensure consistency in the data research method.

Sample size

A sample size of 30 patients were recruited from the period 4 months, from 18 December 2016 to 18 April 2017. The inclusion criteria comprise the location of the pressure ulcers which are limited to the sacrum, buttock and heel as the 3 locations represent over 60% of pressure ulcer wounds and also as a control for confounding factor. The wound length and width were restricted to less than 20cm to cover majority of wounds. This restriction is not due to the scalability of the algorithm. Staging of the wound included stages 2, 3 and 4, however stage 1 wounds are excluded as it only show redness on the skin. The age, gender and race follow the patients profile of HNF. There is no limit to the number of wounds on the patient. A list of patients with wounds were generated from HNF electronic medical record. Those meeting the criteria were filter out for selection.

The HNF nurse in-charge of the patient make an initial contact with the patient or guardian to inform the purpose of the study and perform Abbreviated Mental Test (AMT). For the patient who is no longer communicative and unable to provide informed consent, the guardian will be approached. Participation is voluntary, and refusal would not result in differences in services rendered. For those who score >6 in the AMT, once informed consent obtained, the patient will be enrolled into the study.

During the first visit, the wound care nurse will provide the patient information sheet; which are available in English and Chinese Languages, explaining the current study and patient confidentiality, and seek the patient's signed consent before any measurement can be taken.

Manual Measurement

Tracing perimeter of wound using transparency

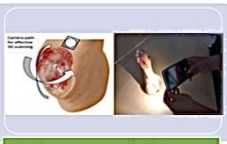
Marking 2 points marked for width (Left) and length (Right) measurement.

Making width (left) and length (right) measurement with a paper ruler

Machine Measurement

Length and width measurement using NDKare 2D photograph

- A square marker with a circular white spot is place on the skin surface that is beside the wound.
- Wound photo was taken and sent to cloud.
- Length and width measurement are formulated by the machine's program based on the 2D photograph of the wound and the square marker as a reference.
- To ensure minimal distorted measurement, photo taking need to be perpendicular to the plane of the wound and marker should lies on the same plane as the wound.



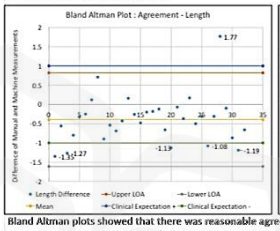
Depth measurement method using video taking method via NDKare™ video capture feature.

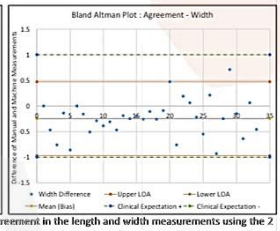
- The square marker should be captured during video taking.
- In landscape mode, within 20 seconds, the wound was captured starting from the left to right, right to center, center to top and top to bottom in a semispherical manner.
- The videos are sent to the cloud to process the measurement
- 2 videos of the same wound were taken so that the intra-rater reliability of the depth measurement can be validated.

RESULTS

Over a 4-month period, 32 wounds were assessed. Demographic of the patients range from age 30 to 96 yrs old. 63% were females and 37% were male. For race, 80% Chinese, 17% Malays and 3% Indians. The wound sizes ranged from 0.5cm² to 29 cm² in area.

1.Results on the Length and Width

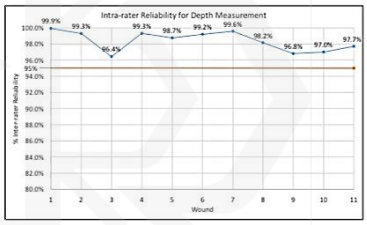




Bland Altman plots showed that there was reasonable agreement in the length and width measurements using the 2 methods with mean difference of -0.3963cm (range -1.35, 1.77) cm and -0.2494cm (range -0.93, 0.71) respectively. The length agreement between manual and machine measurements is 96.9% based on the Bland Altman anaylsis. Out of 32 data points (32 sets of manual and machine measurements), only 1 data point (3.1%) falls out of the Upper Limit of Agreement (mean +2 Standard Deviation). Similarly, for the width.

Postulating that a +/- 1cm tolerance in the differences to be of clinical equivalence between the 2 methods, 100% of the width measurements met the criteria but 6 length measurements exceeded this tolerance.

2.Results on the Depth



To verify the consistency of the depth measured with 3D technology, 2 consecutive videos of the same wound were taken and 2 3D models were generated from the 2 videos. The depth measured from 2 generated 3D models show that depth measured for both 3D models were consistent.

Depth measurement consistency is verified with 11 pairs of validated 3D models (from 11 patients). The low number is due to 4 patients' 3D models were planar (flat surface) and the rest were marker related issues, such as no marker or marker dropped off.

The consistency for depth measurements which was validated by machine has an intra-rater reliability of above 95%.

DISCUSSION & CONCLUSION

1.The result 2D measurement validated the accuracy of the length and width measurement between machine and manual measurements for wounds on sacrum, buttock and heel which are less than 20cm length and width using the Bland Altman Plot.

2.Since length and width measurements are computed by the machine from the 2D photograph, the angle at which the photo is taken and the placement of the square marker such that it is along the same plane of the wound will significantly affect the accuracy. On top of that, it could not compensate for the natural curvature of the body, there were 6 length measurements that exceed the clinical expectation of 1 cm. This is due to the natural curvature of the body on which the wound was measured. These 6 wounds consist of 5 at the sacrum and 1 at the heel, where body curvature is significant. The figure below shows the 3D model of a wound with body curvature, making wound tracing, manual and machine measurements challenging and inconsistent. To overcome this limitation, a length and width measured directly from a 3-dimensional model of the wound surface will provide a more complete representation of the geometry of the wound surface.

IMPLICATION ON PRACTICE

In home nursing practice, the use of manual ruler or wound tracing has been a well-established norm due to the low cost and ease of use. However, there are reliability and accuracy issue with this technique. Thus, a large scale study could look at validating the NDKare machine with another well validated machine. This study is limited by a small sample size. A large scale study could involve a more representative sample size and include patient with deep wound so that we can better validate the accuracy of this solution.

The NDKare solution has proven to have validity in the accuracy for length and width. From users perspectives, there are time saving, ease of use, seamless uploading and trending of wound status into the electronic medical record, and last by not least better photo clarity to facilitate multidisciplinary discussion. Given that manual measurement using paper ruler has inter-rater reliability, the option of adopting this solution is a viable option.

practice

Increasing productivity with smartphone digital imagery wound measurements and analysis

Objective: The primary aim was to determine the productivity increase using digital imagery for better documentation and analysis. A case series was done in a specialised care centre with patients managed with advanced dressings and using state-of-the-art smartphone technology for documentation to save costs and time.
Method: Wounds were cleansed and debrided before using the application to photograph, document, measure and analyse the wounds. The smartphone app was oriented parallel to the plane of the wound, where possible, to obtain accurate measurements.
A longitudinal study report was generated for each wound and showed the progress of the wound healing until the wound was closed.
Results: A sample size of 60 patients consisting of wounds from different locations, and a total of 203 measurements and analyses were conducted over a period of seven months. The wound monitoring app proved to be effective for wound monitoring and required less than two hours' training. A report summary of wounds recorded could also be generated automatically through the dashboard. All 60 patients' cases were automatically recorded, measured and presented into reports for use in clinical analysis. There was a significant time savings (27 hours per day for a specialised care centre with 10 nurses) increase over manual wound documentation and measuring methods.
Conclusion: The app provided a non-contact, easy to use, reliable and accurate smart wound management solution for clinicians and physicians to track wound healing in patients. The app could also be used by patients and caregivers for home monitoring of their wounds.
Declaration of interest: The author has no conflicts of interest.

cost • digital imagery • documentation • productivity • smartphone • time

With the digitalisation drive in Malaysia, the Government is encouraging the use of information technology to improve health-care by reducing costs and improving outcomes. In Malaysia, there is an ever-growing ageing patient population with chronic diseases such as wounds. Diabetes is on the rise, with 17.5% of the population having this debilitating disease.[1] In addition, 25% of these patients develop chronic wound complications. Increased monitoring to assist in identifying complications, customising local wound care treatments and adherence is required. There are many challenges in wound care, including: the lack of standardisation of consolidated wound data, such as a central wound documentation repository where clinicians can readily access well-presented patient wound records; and the high costs of treating wound complications resulting from lack of proper monitoring and fragmented efforts (Fig 1) .

The wound care unit in Hospital Kuala Lumpur (HKL) recognised the need for efficient monitoring of patients' wounds and was approached by the NDKare smart wound management solution development team to evaluate the use of such an application in a wound care centre setting. This application is a smartphone-based point-of-care system that will enable collaboration between medical disciplines, which aims to improve health outcomes, reduce costs and enhance patient experience (Fig 2).[2] A study has reported that it is an intuitive, secure, collaborative, cost-effective and stable wound care management system.[3] It requires a mobile smartphone, installed with the app, with an internet or 4G connection and is available on Android and iOS platform. In addition, it has an effective longitudinal wound report that meets legal and regulatory requirements and thae dashboard that allows clinicians to automatically generate longitudinal wound reports. The smartphone app is a commercially available product.[4]

A two year study (2015–2017), by the Home Nursing Foundation, Singapore, had more than four months' of quantitative data with a wide patient profile showing the reliability and the validity of using this application in patients with wounds.[3].

Aim

The aim of this study was to determine the productivity increase using digital imagery for better documentation and analysis.

Material and methods

The clinical evaluation of the digital application was done at the Wound Care Unit, Hospital Kuala Lumpur (HKL)

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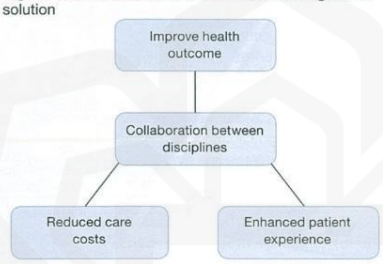
Fig 1. Identified challenges in clinical practice

Fig 2. Potential benefits of smart wound management solution



from January to July 2018 (seven months). Patients were chosen from the population of HKL's wound care unit. Patients that satisfied the inclusion criteria were selected randomly to remove bias from the study. A wound care nurse was assigned to a specified patient, and the same nurse took all the measurements throughout the study to avoid any inconsistencies in use and interpretation of the app. There were three nurses involved in the study and patients fitting inclusion criteria and taken care of by any of the three nurses were enrolled

Training for using the smartphone app was conducted by the company's development team for nurses at the Wound Care Unit at HKL. The nurses were trained to take a single photo of the wound and a video of the wound with the smartphone camera at a fixed distance and at different angles. Proficiency was determined by ensuring the nurses were able to use the app to measure and document the wound effectively. The nurses were able to learn how to use it within two hours.

The basic procedure was that the wound care nurse removed the bandage and cleaned the wound thoroughly, including debridement, before the app was used.

Measurement and documentation

The following protocol was followed:

- Ensure that the patient is comfortably positioned in the anatomically correct position
- Position the marker in the same plane of the wound
- Position the camera apparatus at a standard distance of approximately 5cm from the wound
- Take the photo at a consistent perpendicular angle of the wound
- Take one video of the wound at fixed distance and at different angles

The measurements and photos were stored securely and encrypted on a server maintained and protected by the service provider. Measurements are recorded in the database. Area measurements based on actual area (based on perimeter for the wound) and the area based on length x width were compared.

Inclusion criteria

- External wounds
- Patients with wound length <20cm and width <20cm
- Age (to follow the patient distribution of HKL)
- Gender (to follow the patient distribution of HKL)
- Race (to follow the patient distribution of HKL)
- Number of wounds on the patient (no limit).

Exclusion criteria

- Non-blanchable erythema of intact skin
- Wounds with significant curvature where the marker cannot be placed on the same plane as the wound.

Non-blanchable erythemas were excluded as they appear only as redness on the skin e.g. category 1 pressure ulcers.

The outcome measure was to examine if the time and cost savings in the measurement and documentation of wound could be obtained by using the app.

Results

We recruited 60 patients with a variety of wounds, including DFUs and PUs, from different locations and a total of 203 measurements and analyses were conducted over a period of seven months.
Here we present a selection of cases.

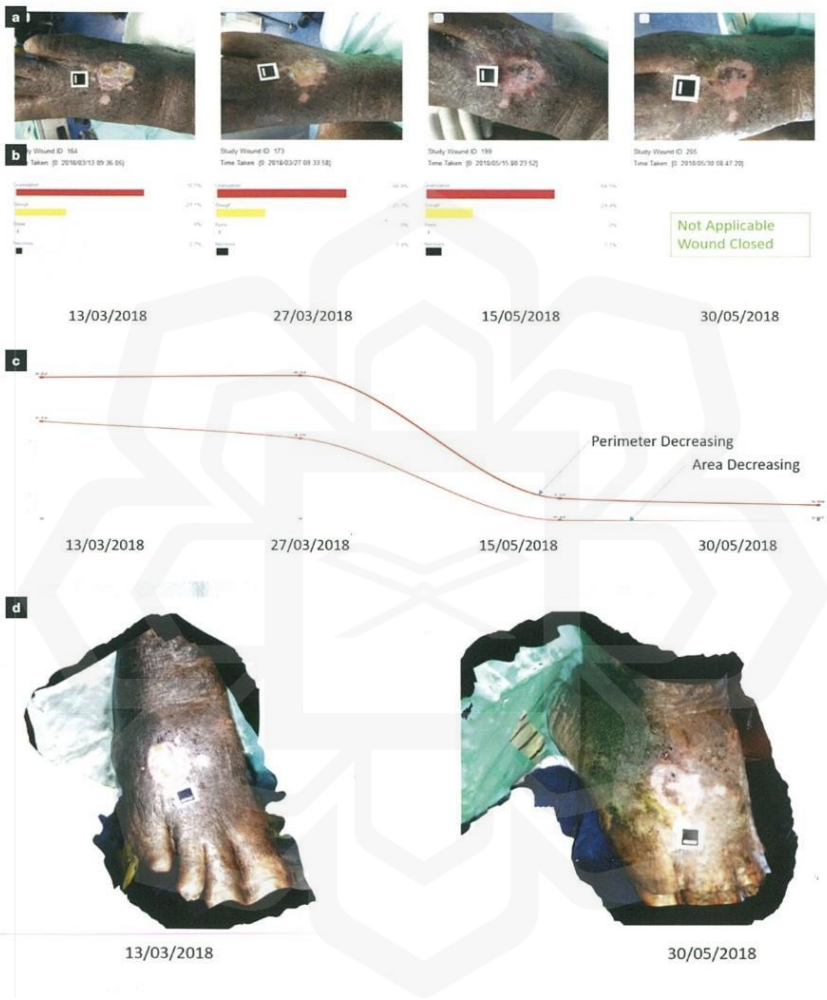
Case 1: diabetic foot ulcer (right foot)

A 92-year-old male with a DFU of the right foot of approximately five months' duration. Comorbidities included chronic venous ulcer and stasis eczema. The wound was treated with stimulen gel (South West Technologies) with PolyMem Pink (PolyMem). Using the app, actual wound area decreased from an initial size of 5.7cm^2 to closure of the wound. The area calculated based on length and width measurements decreased from 7.9cm^2 to closure of the wound (Fig 3).

Case 2: diabetic foot ulcer (left foot)

A 71-year-old male with a DFU of the left foot. The patient had diabetes. The wound was treated with stimulen gel. Using the app, the actual wound area decreased from 7.8cm^2 to closure recorded over approximately 2 months. The area calculated on length and width measurements decreased from 10.8cm^2 to closure (Fig 4).

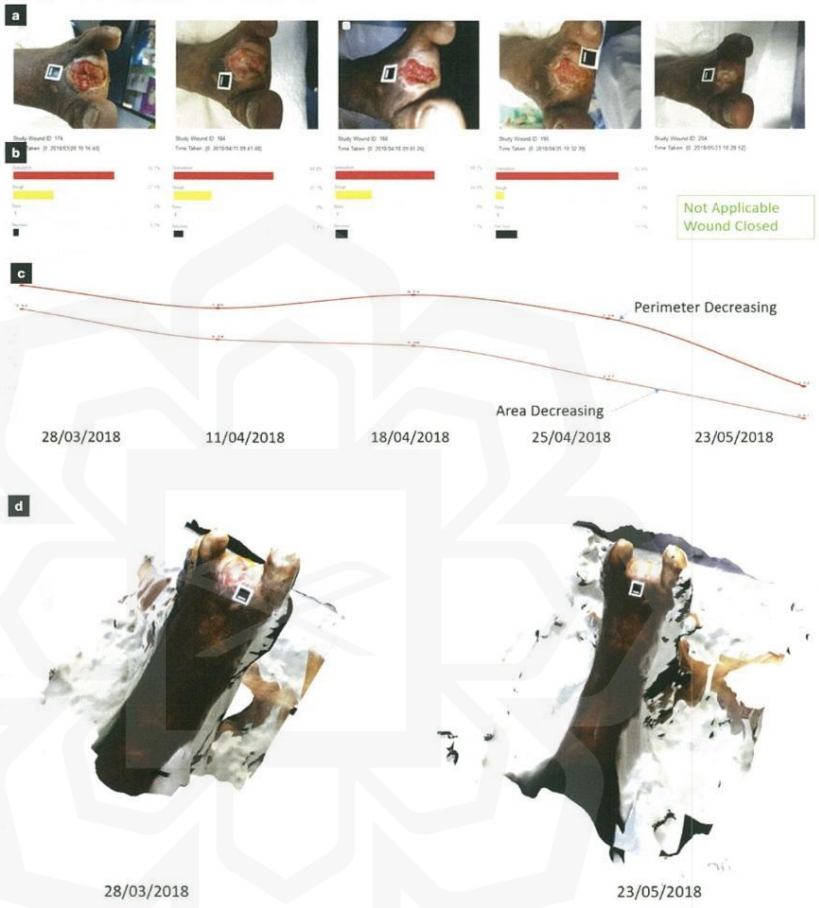
Fig 3. Case 1, diabetic foot ulcer, right foot . Progression of wound healing from initial presentation (13 March 2018) to complete closure (30 May 2018) (**a**). Automatic measurements of granulation, slough, bone and necrosis (**b**). A line graph showing perimeter and area measurements over same timepoints (**c**). Wound at initial presentation and at closure using the apps digital imagery (**d**)



Case 3

A 41-year-old female with a wound on the right thigh. Comorbidities included diabetes and psoriasis anaemia. The wound was treated with MediHoney (Derma Sciences), L-Mesitran Tulle (Mesitran Netherlands), BETAplast N (Mundipharma) and barrier cream. Using the app, the actual area decreased from 99.8cm^2 to closure of the wound. The area calculated based on length and width measurements decreased from 152cm^2 to closure of the wound.

Fig 4. Case 2, a diabetic foot ulcer (left foot). Progression of wound healing from initial presentation (28 March 2018) to complete closure (23 May 2018) (**a**). Automatic measurements of granulation, slough, bone and necrosis (**b**). A line graph showing perimeter and area measurements over same timepoints (**c**). Wound at initial presentation and at closure using the apps digital imagery (**d**)



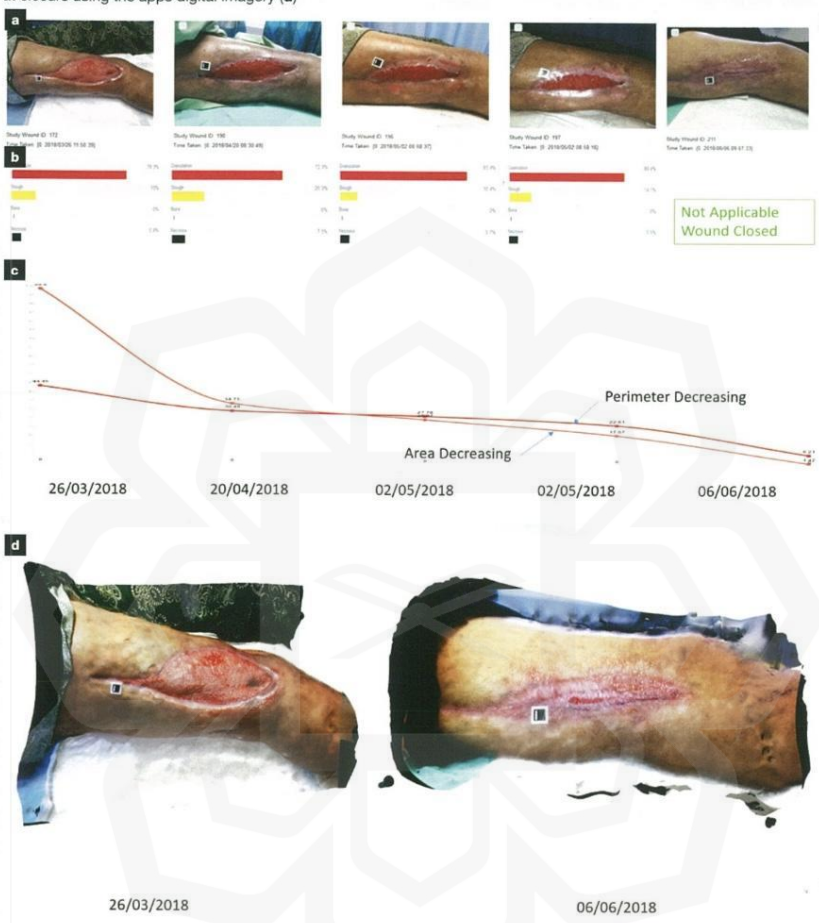
Case 4: diabetic foot ulcer

A 48-year-old male with DFU. The patient was diabetic. The wound was treated with medihoney, BETAplast N, barrier cream, Natrox (Inotec) and stimulant lotion. Using the app, the actual areas of the wound decreased .from an initial 20.3cm^2 to closure of the wound. The area calculated based on length and width measurements decreased from 50.7cm^2 to closure of the wound.

Estimated productivity increase and cost savings

Clinician compensation was based on the average pay of a nurse in Malaysia, estimated at RM3000 Malaysia Ringgit (approximately US$730, £570) (Table 1). The same computation can be adapted to different scenarios and for different countries currencies. This also assumed that each nurse would receive a bonus of one month's pay at the end of the year. There was also no overtime

Fig 5. Case 3, a 41-year-old female with a right thigh wound. Progression of wound healing from initial presentation (26 March 2018) to complete closure (6 June 2018) (**a**). Automatic measurements of granulation, slough, bone and necrosis (**b**). A line graph showing perimeter and area measurements over same timepoints (**c**). Wound at initial presentation and at closure using the apps digital imagery (**d**)



work required of the nurses and the calculation deducted the holidays in a year to compute the average cost per hour, RM20.48 (Table 2).

Full measurement (length, width, area, perimeter, depth and volume) and documentation of the patient's wound was estimated to take 30 minutes. Using the smartphone app, the time taken to measure the wound, was estimated to be at most five minutes. Giving a manpower cost saving per wound measurement of RM8.53 (Table 2)

At the specialised wound care unit at HKL, there are a total of 10 nurses and the number of patients visiting the centre averages 65 patients per day. Based on this the total cost savings per annum were estimated to be RM133,140.76 based on 20 working days per month 12 months a year (Table 2)

Fig 6. Case 4, diabetic foot. Progression of wound healing from initial presentation (13 March 2018) to complete closure (30 May 2018) (**a**). Automatic measurements of granulation, slough, bone and necrosis (**b**). A line graph showing perimeter and area measurements over same timepoints (**c**). Wound at initial presentation and at closure using the apps digital imagery (**d**)

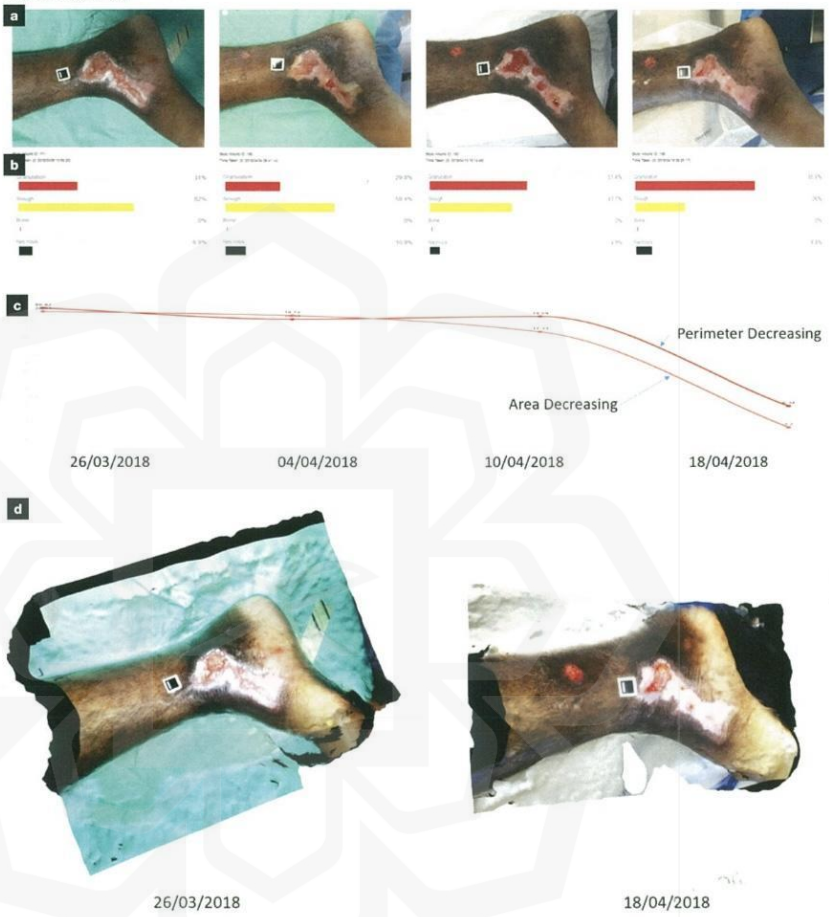


Table 1. Assumptions about clinicians' pay

	Assumptions
1	No over-time compensation
2	Average salary of RM3000 (in Malaysian ringgit)
3	Bonus of one month's pay in addition to monthly pay
4	Exclude compliance costs

Each nurse undertook the following processing starting with registering the patient, preparing the patient, assessing the patient, cleaning the wound, measuring/documenting the wound, treating and bandaging the wound.

The savings estimated only considers the time savings for nursing staff. There will be further savings for other office staff, such as registration receptionists and assistants that is not included in this calculation.

Due to the time savings, it was possible for the wound care unit to perform up to 325 wound measurements and documentations per day in the specialised care centre, as shown in Table 3.

Discussion

In the Wound Care Unit, Dept of Internal Medicine, the use of a manual ruler or wound tracing has been a well-established norm due to the low cost and ease of use. However, there are reliability and accuracy issues with this technique. Therefore, a pilot study was conducted with the smartphone application over a two-month period which showed the practicality and ease of use of this application in the overall management of the wounds.[4]

The smartphone application has proven to have validity in accuracy for length, width, area and perimeter. From the clinicians' perspective, there are time savings, productivity savings of 500%, ease of use, seamless trending of wound progress into the electronic medical record and, last but not least, better photographic clarity to facilitate multidisciplinary discussion.

Limitations

This study only assessed the use of the app to measure wounds; this was not compared with traditional methods for comparative analysis. Furthermore, the time taken for measurement was an estimation based on our experience rather than actual timing, although we believe these are consistent in our experience.

Conclusion

The non-invasive nature of this application also improved quality and consistency with, a reduction in errors in clinical wound-care documentation. Adoption of this solution would reduce costs and improve workflow. Applications such as this are beneficial in terms of better financial management, ability to track return on investment and variable costs, and as a collaborative mobile platform whereby there is remote accessibility and ease of collaboration between disciplines. **JWC**

Table 2. Total cost savings per annum for specialised wound care centre at Hospital Kuala Lumpur

Cost per hour				
Hours	**Week**	**Cost of a registered nurse (RM3000 x 13 months)**	**39,000**	**per annum**
42	52	1 nurse works 42 hour/week (52 weeks per year)	2184	hours/year
Days	**Hours/day**			
21	8	Subtract 21 days annual leave (8 hour per day)	168	hours/year
11	8	Subtract 11 days public holidays (8 hours per day)	88	hours/year
3	8	Buffer three days for family care leave, medical leave	24	hours/year
		Net work hours per nurse	1904	hours/year
		Cost per hour	**RM20.48**	**per hour**
Time and cost savings per patient				
Mins	**Manpower cost saving app versus manual measure**			
30	Manpower cost for manual wound measurement			RM10.24
5	Manpower cost for wound measurement using app			RM1.71
	Manpower cost savings per wound measurement using app versus manual			RM8.53
Patients per day for specialised care centre at Hospital Kuala Lumpur				
65	Manpower cost for 65 patients per day (assuming one wound per patient)			RM554.75
Working days				
20	Cost savings per month (20 working days)			RM11,095.06
Cost savings/year (20 working days/month x 12 months)				**RM133,140.76**

Table 3. Productivity increase in time

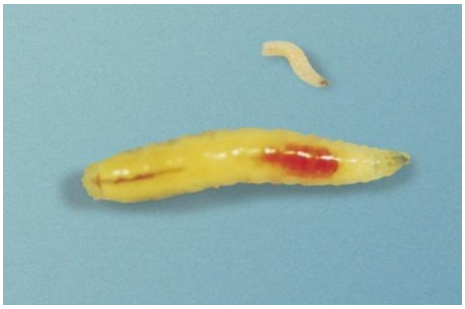
Productivity	**Per day**		
	No. of patients	**No. of nurses**	**Time needed (min)**
Status quo	65	10	1950
Wound app	65	10	325
Total time difference (δ)			1625
% productivity increase			500% #3
Wound measurement unit can take up to 325 patients			

References

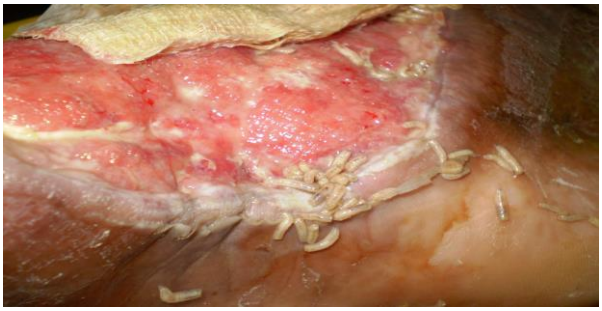
1 National Health Morbidity Study 3, Ministry of Health Malaysia; 2015
2 Langemo D, Anderson J, Hanson D, Hunter S, Thompson P. Measuring wound length, width, and area: which technique? Advances in Skin & Wound Care, January 2008, 21(1): 42-45
3 Perspective cohort study to validate the accuracy and repeatability of non-contact pressure ulcer wound measurement published by National Home Nursing foundation of Singapore and validated by Biostatistics Unit, Yong Loo Lin School of Medicine, National University Health System, Singapore
4 Hospital Kuala Lumpur report (Clinical evaluation conducted over 2 months period from Jan 2018 to Mar 2018)

APPENDIX C

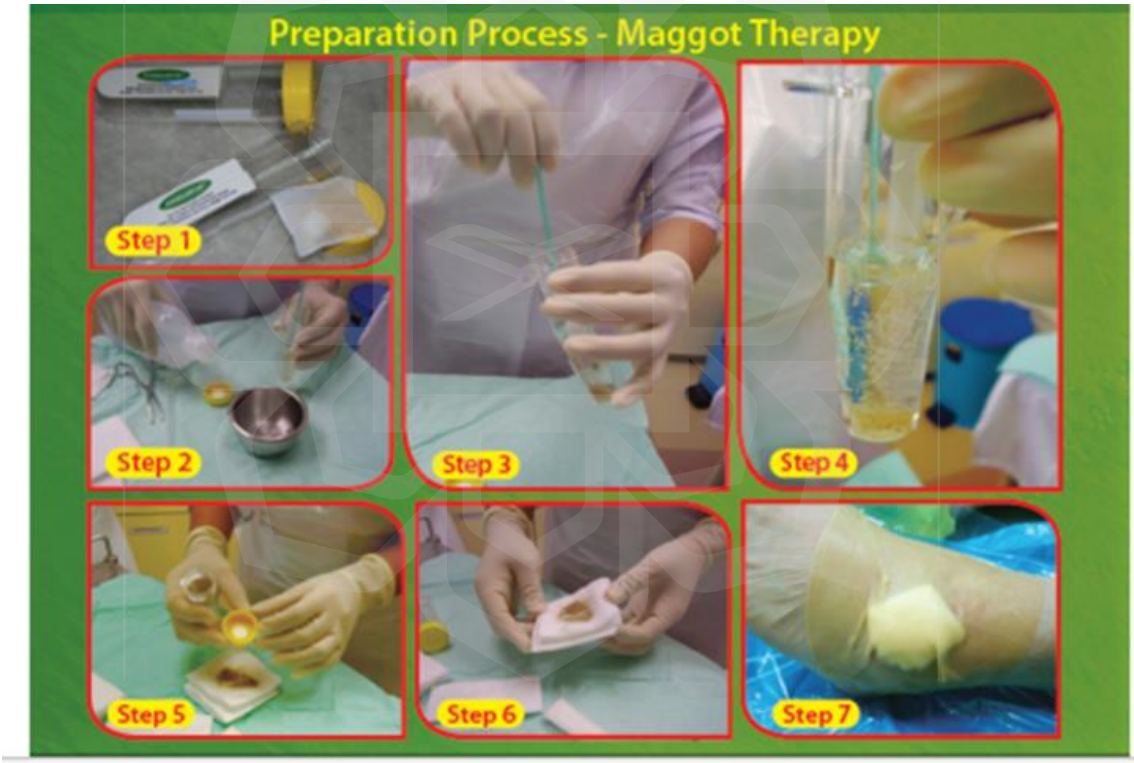
ILLUSTRATION FOR MAGGOTS ON THE WOUND, IMAGE OF MAGGOT DURING APPLICATION & AFTER 72HOURS & MDT APPLICATION PROCESS



Size of maggots on the day of application & after 72 hours



Maggots crawling on the wound after 72 hours



Maggot preparation process prior to application

APPENDIX D

CERTIFICATE OF ANALYSIS FOR KELULUT HONEY FROM HUKM

UKM UNIPEQ

Ref : ULUKM/10191/18
Date : 23/10/2018
Page : 1 of 1

CERTIFICATE OF ANALYSIS

Name of Customer : **NALURI PANTAS SDN BHD**(1212836-U)
Address : No 10, Jalan Rimba Riang 9/8, Seksyen 9, Kota Damansara, 47810 Petaling Jaya, Selangor.
Tel. No. : 012-321 2760
Sample Description : ***Madu Kelulut – Dino Kelulut***
Sample Ref. No : U2664/18
Date of Receipt : 02/10/2018

ANALYSIS RESULTS
(As per sample)

Parameter, Unit	Result	MS 2683:2017	Test Method
Ash, g/100g	0.6	<1.0	In-house Method No: STP/Honey/02- based on Harmonised Methods of International Honey Commission 1.3
Moisture, g/100g	30.7	<35.0	In house method No. STP/ Honey/04 based on AOAC 16th Edi. 969.38 & 925.45
Reducing Sugar, g/100g - Fructose - Glucose	 5.5 5.2	 <85	Harmonised Methods of The International Honey Commission, 1.7.2 / HPLC
Maltose, g/100g	N.D (<0.001)	<9.5	Harmonised Methods of The International Honey Commission, 1.7.2 / HPLC
Sucrose, g/100g	4.4	<7.5	Harmonised Methods of The International Honey Commission, 1.7.2 / HPLC
Hidroxymethyl furfural, mg/kg	N.D (<0.1)	<30.0	Harmonised Methods of The International Honey Commission 1.6.1
pH	3.33	2.5-3.8	In-house Method No: STP/Honey/03- based on AOAC Official Method 16th Edition 962.19
Diastase Activity, DN	5.2	-	Harmonised Methods of The International Honey Commission 1.6.1

Remarks:

a. < : Limit of reporting
b. *Opened / balance samples will be discarded two weeks after issuance of Certificate of Analysis.*

NOOR AZINAH MAAMIN
CHEMIST (M.M.I.C)
IKM No. M/1973/4360/03

Bridge To A Better Product UNIPEQ Sdn. Bhd. (870956-D) Block A, UKM - MTDC Technology Centre, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan Malaysia
Tel : 603 8921 5965 Fax : 603 8925 2115 Email : unipeq@ukm.edu.my Web : www.unipeq.com.my

APPENDIX E

INTERNAL APPROVAL LETTER FOR DATA COLLECTION IN UMMC

UNIVERSITI MALAYA

Ruj. Kami : PPUM/RDI/600/10/001/03

4 Rabi'ulakhir 1443H
9 November 2021

Dr. Nik Aizah Nabilla Binti Faheem
Jabatan Surgeri Ortopedik
Pusat Perubatan Universiti Malaya

Puan,

Kebenaran Untuk Menjalankan Penyelidikan Di Pusat Perubatan Universiti Malaya

Dengan hormatnya saya merujuk kepada perkara di atas.

2. Sukacita dimaklumkan bahawa permohonan untuk menjalankan penyelidikan seperti dibawah adalah diluluskan dengan syarat hanya Doktor dan jururawat yang bertugas boleh melakukan proses *debridement* dan membalut luka. Pihak penyelidik juga diingatkan untuk mematuhi syarat bahawa data hanya boleh diakses daripada jangka masa yang dimohon serta sebarang penerbitan perlulah menghantar *'softcopy'* kepada pihak Jabatan Penyelidikan, Pembangunan dan Inovasi.

3. Maklumat penyelidikan adalah seperti berikut :

Nama Penyelidik	:	1) Pirehma a/p Marimuthu
Tajuk Penyelidikan	:	*The Efficacy Of Kelulut Honey As Compared To Medical Grade Manuka Honey In The Treatment Of Diabetic Foot Ulcer (Post-Debrided With Maggot Therapy*
Tarikh Penyelidikan	:	1/10/2021 – 1/10/2023

4. Dengan kebenaran ini diharap dapat menjalankan penyelidikan dengan penuh integriti dan dapat menghasilkan penyelidikan yang berkualiti dan berimpak tinggi.

Sekian, Terima kasih.

"PENERAJU PENGAJARAN PERUBATAN"

"WAWASAN KEMAKMURAN BERSAMA 2030"

"BERKHIDMAT UNTUK NEGARA"

Saya yang menjalankan amanah,

PROFESOR DR. NAZIRAH BINTI HASNAN
Pengarah
Pusat Perubatan Universiti Malaya

APPENDIX F

RESEARCH MODULE CERTIFICATE FOR THE CREDENTIAL & PRIVILEGING

JPPIC&PR000000007/2021

UNIVERSITY OF MALAYA
MEDICAL CENTRE

RESEARCH MODULE CERTIFICATE

This certifies that

PIREHMA MARIMUTHU

has successfully completed online research module as a requirement of Credential & Privileging for Research

Dated : 05 October 2021

PROF. DR. MAZNAH BINTI DAHLUI

Chairman of Research Management Committe
University Malaya Medical Centre

APPENDIX G

MREC APPROVAL LETTER

UNIVERSITY OF MALAYA MEDICAL CENTRE
MEDICAL RESEARCH ETHICS COMMITTEE
(Formerly known as Medical Ethics Committee)
UNIVERSITY OF MALAYA MEDICAL CENTER
ADDRESS : LEMBAH PANTAI, 59100 KUALA LUMPUR, MALAYSIA
TELEPHONE : 03-79493209/2251 FAXIMILE : 03-79492030

NAME OF ETHICS COMMITTEE/IRB Medical Research Ethics Committee, University Malaya Medical Center	**MRECID.NO**: 2021420-10082
ADDRESS : LEMBAH PANTAI, 59100 KUALA LUMPUR, MALAYSIA	**NMRR.ID :** NMRR-21-1653-61162
PROTOCOL NO(if applicable) :	
TITLE: THE EFFICACY OF KELULUT HONEY AS COMPARED TO MANUKA HONEY IN THE TREATMENT OF DIABETIC FOOT ULCER (POST-DEBRIDED WITH MAGGOT THERAPY)	
PRINCIPAL INVESTIGATOR : Dr Nik Aizah Nabilla Bt Faheem	**SPONSOR** -

The following item [✓] have been received and reviewed in connection with the above study to conducted by the above investigator.

[✓]	Application to Conduct Research Project(form)	Ver.No :	Ver.Date : 20-04-2021
[✓]	Study Protocol	Ver.No : 3	Ver.Date : 17-07-2021
[✓]	Patient Information Sheet	Ver.No : 2	Ver.Date : 04-05-2021
[✓]	Consent Form	Ver.No : 2	Ver.Date : 04-05-2021
[ ]	Questionnaire	Ver.No :	Ver.Date :
[✓]	Investigator's CV / GCP (Dr Nik Aizah Nabilla Bt Faheem,PIREHMA MARIMUTHU,)	Ver.No :	Ver.Date :
[ ]	Insurance certificate	Ver.No :	Ver.Date :
[✓]	Other Attachments		
	1) APPROVAL LETTER FOR THE PROPOSED STUDY FROM UIAM	Ver.No : -	Ver.Date :
	2) NDKare app	Ver.No : -	Ver.Date :
	3) literature review on honey(manuka,kelulut)	Ver.No : -	Ver.Date :
	4) literature review maggot	Ver.No : -	Ver.Date :
	5) manuka honey_clinical use	Ver.No : -	Ver.Date :
	6) kelulut honey_cert of analysis	Ver.No : -	Ver.Date :
	7) animal study with kelulut honey_UPM	Ver.No : -	Ver.Date :
	8) Data Collection Sheet	Ver.No : -	Ver.Date :
	9) safety reporting	Ver.No : -	Ver.Date :

and the decision is [✓]

[✓] Approved
[ ] Expedited approved
[ ] Rejected(reasons specified below or in accompanying letter)

Comments:

APPROVE.

Investigator are required to:

1) *follow instructions, guidelines and requirements of the Medical Research Ethics Committee.*
2) *report any protocol deviations/violations to Medical Research Ethics Committee.*
3) *provide annual and closure report to the Medical Research Ethics Committee.*
4) *comply with International Conference on Harmonization – Guidelines for Good Clinical Practice (ICH-GCP) and Declaration of Helsinki.*
5) *obtain a permission from the Director of UMMC to start research that involves recruitment of UMMC patient.*
6) *ensure that if the research is sponsored, the usage of consumable items and laboratory tests from UMMC services are not charged in the patient's hospital bills but are borne by research grant.*
7) *note that he/she can appeal to the Chairman of Medical Research Ethics Committee for studies that are rejected.*
8) *note that Medical Research Ethics Committee may audit the approved study.*
9) *ensure that the study does not take precedence over the safety of subjects.*

Date of meeting : 28-07-2021
Date of approval : 28-07-2021
Approval By : LOOI LAI MENG (Chairman,MREC)

This is a computer generated letter. No signature required.

APPENDIX H

PATIENT INFORMATION CONSENT FORM

KEIZINAN OLEH PESAKIT UNTUK PENYELIDIKAN KLINIKAL

Nombor Versi: 2
Tarikh Versi: 04/05/2021

Saya,.....No Kad Pengenalan.....
(Nama Pesakit)

beralamat.....
(Alamat)

dengan ini bersetuju menyertai dalam penyelidikan klinikal (pengajian klinikal/pengajian soal-selidik/percubaan ubat-ubatan) disebut berikut:

TajukPenyelidikan: THE EFFICACY OF KELULUT HONEY AS COMPARED TO MANUKA HONEY FOR THE TREATMENT OF DIABETIC FOOT ULCER (POST-DEBRIDED WITH MAGGOT THERAPY)

yang mana sifat dan tujuannya telah diterangkan kepada saya oleh Dr.....
(Nama & Jawatan Doktor)

mengikut terjemahan..... yang telah menterjemahkan kepada saya
(Nama & Jawatan Penterjemah)

dengan sepenuh kemampuan dan kebolehannya di dalam Bahasa / loghat.....

Saya telah diberitahu bahawa dasar penyelidikan klinikal dalam keadaan methodologi, risiko dan komplikasi (mengikut kertas maklumat pesakit). Selepas mengetahui dan memahami semua kemungkinan kebaikan dan keburukan penyelidikan klinikal ini, saya merelakan/mengizinkan sendiri menyertai penyelidikan klinikal tersebut di atas.

Saya faham bahawa saya boleh menarik diri dari penyelidikan klinikal ini pada bila-bila masa tanpa memberi sebarang alasan dalam situasi ini dan tidak akan dikecualikan dari kemudahan rawatan dari doktor yang merawat.

Tarikh: ..... Tandatangan/Cap Jari .....
(Pesakit)

DI HADAPAN

Nama .....)

No. K/P.....) Tandatangan .....
(Saksi untuk Tandatangan Pesakit)

Jawatan.....)

Saya sahkan bahawa saya telah menerangkan kepada pesakit sifat dan tujuan penyelidikan klinikal tersebut di atas.

Tarikh: ..... Tandatangan .....
(Doktor yang merawat)

KEIZINAN OLEH PESAKIT UNTUK PENYELIDIKAN KLINIKAL	No. Pendaftaran Nama Jantina Umur Unit		**BK-MIS-1117-E02**

CONSENT BY PATIENT FOR CLINICAL RESEARCH

Version No.: 2
Version Date: 04/05/2021

I, ……. Identity Card No……
(Name of Patient)
of
……
……..
(Address)
hereby agree to take part in the clinical research (clinical study/questionnaire study/drug trial) specified below:

Title of Study: THE EFFICACY OF KELULUT HONEY AS COMPARED TO MANUKA HONEY FOR THE TREATMENT OF DIABETIC FOOT ULCER (POST-DEBRIDED WITH MAGGOT THERAPY)

the nature and purpose of which has been explained to me by Dr ……
(Name & Designation of Doctor)

and interpreted by …… to the best of his/her ability in
(Name & Designation of Interpreter)
…… language/dialect.

I have been told about the nature of the clinical research in terms of methodology, possible adverse effects and complications (as per patient information sheet). After knowing and understanding all the possible advantages and disadvantages of this clinical research, I voluntarily consent of my own free will to participate in the clinical research specified above.

I understand that I can withdraw from this clinical research at any time without assigning any reason whatsoever and in such a situation shall not be denied the benefits of usual treatment by the attending doctors.

Date: …… Signature or Thumbprint ……
(Patient)

IN THE PRESENCE OF

Name ……)
)
Identity Card No. …… Signature ……
(Witness for Signature of Patient)
Designation ……)

I confirm that I have explained to the patient the nature and purpose of the above-mentioned clinical research.

Date …… Signature
……
(Attending Doctor)

CONSENT BY PATIENT FOR CLINICAL RESEARCH	R.N. Name Sex Age Unit		**BK-MIS-1117-E02**

CONSENT BY RESPONSIBLE RELATIVE FOR CLINICAL RESEARCH

Version No.: 2
Version Date: 04/05/2021

I, ... Identity Card No.....
(Name)
of
.....
(Address)

hereby agree that my relative ..... I.C. No.....
(Name)
participate in the clinical research (clinical study/questionnaire study/drug trial) specified below:-

Title of Study: THE EFFICACY OF KELULUT HONEY AS COMPARED TO MANUKA HONEY FOR THE TREATMENT OF DIABETIC FOOT ULCER (POST-DEBRIDED WITH MAGGOT THERAPY)

the nature and purpose of which has been explained to me by Dr
.....
(Name & Designation of Doctor)

and interpreted by .....
(Name & Designation of Interpreter)

..... to the best of his/her ability in ..... language/dialect.

I have been informed of the nature of this clinical research in terms of procedure, possible adverse effects and complications (as per patient information sheet). I understand the possible advantages and disadvantages of participating in this research. I voluntarily give my consent for my relative to participate in this research specified above.

I understand that I can withdraw my relative from this clinical research at any time without assigning any reason whatsoever and in such situation, my relative shall not be denied the benefits of usual treatment by the attending doctors. Should my relative regains his/her ability to consent, he/she will have the right to remain in this research or may choose to withdraw.

Date: ..... Relationship to Patient ..... Signature or Thumbprint .....

IN THE PRESENCE OF

Name .....)
)
Identity Card No. .....)
.....
)
Designation .....)

(Witness)

I confirm that I have explained to the patient's relative the nature and purpose of the above-mentioned clinical research.

APPENDIX I

PATIENT INFORMATION SHEET

UNIVERSITY OF MALAYA MEDICAL CENTRE

PARTICIPANT INFORMATION SHEET

Study Title: THE EFFICACY OF KELULUT HONEY AS COMPARED TO MANUKA HONEY IN THE TREATMENT OF DIABETIC FOOT ULCER (POST-DEBRIDED WITH MAGGOT THERAPY)

Version No: 2

Version Date: 04/05/2021

We would like to invite you to take part in a research study. Before you decide whether to participate, you need to understand why the research is being done and what it would involve. Please take time to read the following information carefully; talk to others about the study if you wish.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

---

1. **What is the purpose of this study?**
   To evaluate and compare the efficacy of kelulut honey and manuka honey in the treatment of diabetic foot ulcer (post debrided with maggot therapy)

2. **Why is this study important?**
   Since the number of foot complications and lower limb amputations are on the rise, improving healing rates of diabetic foot ulcers are crucial. In recent times,

manuka honey is utilised for wound treatment due to its antibacterial, anti-inflammatory and anti-oxidant properties. Despite having higher anti-oxidant properties, studies on stingless bee honey locally known as kelulut honey is lacking & comparison studies remains elusive. Thus, the outcome this study is expected to demonstrate is that kelulut honey is more effective in shortening healing time of diabetic foot ulcers and reducing foot complications.

3. What type of study is this?
Experimental study.

4. What is the procedure that is being tested? (If applicable)
Wound dressing and debridement of diabetic foot wounds.

5. Does the investigatory product contain cultural sensitive ingredients eg: bovine or porcine? (if applicable)
No

6. Why have I been invited to participate in this study?
You have been invited to participate in this study because the fulfilment of the inclusion criteria as below:

a. 18 years old and above.

b. Have non healing diabetic foot ulcer.

c. Ulcer do not require urgent debridement.

d. You are not having life threatening infections.

e. Your wound is suitable to use maggot and honey to speed up healing.

7. Who should not participate in the study?
You should not participate in the study if you:

a. Have non diabetes-based foot ulcerations.
b. Have life threatening infections.
c. Need amputation urgently.
d. Need urgent debridement.
e. Have diabetes with non-sloughy wounds (clean wounds).

8. Can I refuse to take part in the study?

Yes, participation in this study is entirely your own right.

9. What will happen to me if I take part?

You are expected to go through treatment with maggot debridement therapy and subsequently honey dressing to speed up healing of your ulcer.

For the maggot debridement therapy, sterile fly larvae are applied directly onto the wound and kept in place using a special dressing system. The exact number of larvae is determined by the size of the wound. The maggot application is generally left in place for three days, and the maggot therapy dressing is then removed, and wound inspected. Re-application may be considered if the wound bed is still sloughy, until the desired effect is achieved.

Subsequently, you will be randomised to receive wound dressing treatment with either kelulut or manuka honey, and you will be monitored for wound healing for up to two weeks after maggot debridement.

10. How long will I be involved in this study?

3 weeks.

11. What are the possible disadvantages and risks?

For the maggot debridement therapy, you may feel a slight uneasiness or discomfort like 'ants crawling' or mild pain. If the pain is unbearable, pain killers will be prescribed by the doctor. If the pain is not controlled, then you may opt for removal of the larvae early.

Sometimes a wound that contains a lot of dead tissue will develop a characteristic smell during treatment. This is nothing to worry about, it is just due to the activity of the larvae and should disappear when the dressing is changed.

The wound may also become a little wetter than usual and show the presence of a dark red or pink discharge. This is also due to the action of the larvae breaking down dead tissue and should not be worried about.

The maggots will NOT bury into your tissue. It only digests dead tissue and does not disturb healthy tissue. The maggots also will NOT multiply in your wound, only adult flies can lay eggs that hatch into maggots.

The instinctive behavior of maggots is to leave the wound once they are finished working (satiated). Most maggots will be satiated by 48 hours, and the remaining are satiated up to 72 hours. The satiated maggots may attempt to escape, and this

may be possible if the dressing comes loose. However, if the dressing is removed earlier than 48 hours, some maggots may still be hiding in any dead tissue, and will escape the wound within the following 24 hours once they are satiated. Thus, you may still find remaining maggots in your subsequent normal dressing, if the maggot dressing had been removed too early.

There are no known disadvantages or risks associated with dressing with manuka or kelulut honey.

12. What are the possible benefits to me?

a. Subsidized treatment with advanced wound therapy such as maggot debridement & honey dressing.
b. Faster healing.
c. Reduce hospital stay.
d. Prevent foot complications.

13. Who will have access to my medical records and research data?

Researcher and principal investigator.

14. Will my records/data be kept confidential?

Yes.

15. What will happen to any samples I give? (If applicable)

No samples are taken for this study.

16. What will happen if I don't want to carry on with the study?

You can withdraw from this study by informing the investigators at any point of time.

17. What if relevant new information about the procedure/ drug/ intervention becomes available? (If applicable)

Not applicable.

18. What happens when the research study stops? (If applicable)

Not applicable.

19. What will happen to the results of the research study?

The results will be compiled and written up for the fulfilment of a PhD degree as well as published in wound journals.

20. Will I receive compensation for participating in this study?

No. The maggot debridement therapy and honey dressing will be free of charge.

21. Who funds this study?

Self-funded by researcher.

22. Who should I contact if I have additional questions/problems during the course of the study?

You may contact us at the following numbers:

Pirehma Marimuthu
PhD candidate in Bio Behavioral Health Sciences
International Islamic University Malaysia
Telephone number: 012-3529783

Nik Aizah Nabilla Bt Faheem
Orthopaedic Surgeon
University of Malaya Medical Center
Telephone number: 017-6727479

23. Who should I contact if I am unhappy with how the study is being conducted?

Medical Research Ethics Committee
University of Malaya Medical Centre
Telephone number: 03-7949 3209/2251

BK-MIS-1116-E03

APPENDIX J

RESEARCH METHODOLOGY & STATISTICAL ANALYSIS VERIFICATION FROM UMMC

UNIVERSITI MALAYA

3 March 2023

Department of Bio Behavioral Science,
Kuliyyah of Nursing,
International Islamic University Malaysia,
Kuantan, Pahang.

Sir/Madam,

Verification of Using The Research Methodology and Statistical Consultation (RMSC) Service

We refer to the matter above.

We would like to inform that the named client below had sought statistical consultation from the Research Methodology and Statistical Consultation (RMSC) service, in the Faculty of Medicine, Universiti Malaya.

Name of client	: Pirehma Marimuthu
Project title	: The efficacy of kelulut honey compared to medical-grade manuka honey in the treatment of diabetic foot ulcer (post-debrided with maggot therapy)
Consultation date	: 22 December 2022 and 13 January 2023 (Total of 3 hours)
Name of consultant	: Dr Lee Zheng Yii

All efforts are made to ensure professional advices or recommendations given by our consultant to the client are accurate based on the information and date provided during the consultation sessions. Our consultant bears no responsibility for the accuracy of the client's statistical analysis or reported findings arising after the consultation services ended.

Thank you.

Yours sincerely,

MOHD ZAMRIEE BIN MUSTAFFAR
Assistant Registrar
Research Training Unit (RTU)
Faculty of Medicine, UM

FACULTY OF MEDICINE
Universiti Malaya, 50603 Kuala Lumpur, MALAYSIA

Tel: (603) 7947 4941 / 3796 / 3146 / 6695 • Fax: (603) 7947 6684 • Website: http://medicine.um.edu.my

APPENDIX K

THESIS PROOFREAD CERTIFICATE

Our reference: **CERT-231023-03UJPirehma**

Proofreaders United

Certificate of Acknowledgement

(Proofreading)

We certify that the following Thesis/Dissertation (MS Word)

COMPARATIVE EFFICACY OF STINGLESS BEE (KELULUT) HONEY AND MEDICAL-GRADE MANUKA HONEY FOLLOWING MAGGOT DEBRIDEMENT THERAPY IN DIABETIC FOOT ULCER TREATMENT

Pirehma Marimuthu

has been proofread by a proofreader from Proofreaders United. The text has been checked for grammar, spelling, and punctuation. Document length: 149.5 page(s) based on our format (Times New Roman, font size 12, 2.0 line spacing, without figures and tables). Full details of the proofreading service can be found at ProofreadersUnited.com.

26 March 2024

Acknowledgement by:

DR. MUHAMMAD ZAKI RAMLI
FOUNDER OF PROOFREADERS UNITED

APPENDIX L

ENGLISH TO ARABIC TRANSLATION VERIFICATION

Our reference: **CERT-251023-03UJPirehma**

Proofreaders United

Certificate of Acknowledgement

(Translation)

We certify that the following Arabic abstract translation verification

COMPARATIVE EFFICACY OF STINGLESS BEE (KELULUT) HONEY AND MEDICAL-GRADE MANUKA HONEY FOLLOWING MAGGOT DEBRIDEMENT THERAPY IN DIABETIC FOOT ULCER TREATMENT

Pirehma Marimuthu

has been translated from English to Arabic by a professional translator from Proofreaders United. Full details of the translation service can be found at ProofreadersUnited.com.

27 March 2024

Acknowledgement by:

MUHAMMAD ZAKI RAMLI
FOUNDER OF PROOFREADERS UNITED

`

APPENDIX M

TURNITIN SIMILARITY REPORT

turnitin plagirsm check

ORIGINALITY REPORT

9%	7%	3%	3%
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5	orca.cf.ac.uk Internet Source	1%

APPENDIX N

PUBLICATIONS

Potential of Malaysian Stingless Bee Kelulut Honey in Wound Healing

M. Pirehma Marimuthu[1], Aniawanis Makhtar [2], Norlinda Abd Rashid [3], Nik Aizah Nabila Faheem [4], Amber Haseeb [5]

[1, 2, 3] International Islamic University Malaysia, Kuantan, Pahang, Malaysia.
[4, 5] University Malaya Medical Centre, Kuala Lumpur, Malaysia.
Email: [1] pirehma@gmail.com, [2] aniawanis@iium.edu.my, [3] anatomi@iium.edu.my, [4] aizahn@ummc.edu.my, [5] amberhaseeb@ummc.edu.my

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COMPARATIVE STUDY BETWEEN MALAYSIAN STINGLESS BEE KELULUT HONEY AND MEDICAL-GRADE MANUKA HONEY IN THE TREATMENT OF DIABETIC FOOT ULCER

M. Pirehma Marimuthu[1], Aniawanis Makhtar[2],Norlinda Abd Rashid[3], Nik Aizah Nabila Faheem[4], Amber Haseeb[5]
[1,2,3] International Islamic University Malaysia, Kuantan, Pahang, Malaysia,
[4,5] University Malaya Medical Centre, Kuala Lumpur
E-Mail: [1]pirehma@gmail.com,[2]aniawanis@iium.edu.my, [3]anatomi@iium.edu.my, [4]aizahn@ummc.edu.my, [5]dramber84@gmail.com

Int. J. Adv. Res. Biol. Sci. (2022). 9(3): 80-94

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Study Protocol: Efficacy of Stingless-Bee Kelulut Honey Dressing Compared To Medical-Grade Manuka Honey In The Treatment Of Diabetic Foot Ulcer

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A Review
The Role of Maggot Debridement Therapy in Wound Healing

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