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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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Abstract

Background: The increasing prevalence of diabetes has contributed largely to the incidences of diabetic foot ulcerations worldwide. Chronic wound such as diabetic foot ulcer (DFU) takes longer time to heal with conventional treatment, gets infected, resistant to antibiotics, and leads to gangrene, even lower limb amputations. Managing DFU has become a challenge for clinicians and a serious healthcare burden with devastating consequences for the patients. The evolution in chronic wound management has led to the resurgence of traditional remedy like honey to improve healing outcomes and reduce foot complications. Topical dressing using medical-grade manuka honey had shown positive healing outcomes due to its antibacterial, anti-inflammatory and antioxidant properties. However, research on stingless-bee honey locally known as kelulut honey has yet to progress into significant wound healing evaluations despite having higher antioxidant properties. Thus, this study is aimed to evaluate the efficacy of kelulut honey as compared to medical-grade manuka honey in the treatment of DFU.

Method: This experimental study will include 60 participants recruited in a tertiary teaching hospital, University Malaya Medical Centre (UMMC). All sloughy DFU will be debrided with maggot therapy and divided into Group 1(intervention group treated with kelulut honey) and Group 2 (control group treated with medical-grade manuka honey). Follow up will be done for 2 weeks with wound measurement at baseline, day 7 and 14 to determine the healing rate.

Discussion: The outcome of the study may provide the much-needed clinical evidence for kelulut honey to be considered for wound healing and future research could establish kelulut honey as an alternative topical dressing for DFU.

Keywords: chronic wound, diabetic foot ulcer, wound healing, honey, stingless bee

Background

Prevalence of diabetes and incidences of diabetes related complications has been on the rise across the globe for the past few decades as ageing population increases and sedentary lifestyle display its dominancy (Al-Rubeaan et al., 2015; Cho et al., 2018; Zhang et al., 2017). One of the main devastating complication of diabetes is poor healing of foot ulcerations which largely contributes to lower limb amputations (Hinchliffe et al., 2016). Diabetic foot ulcer (DFU) has been categorized as a chronic wound as it has been vastly reported not to heal in a timely manner within 3 months with the standard or conventional treatment (Chun et al., 2019; Noor, Khan, & Ahmad, 2017; Syafril, 2018). Much has been reported about the risk factors for non-healing DFU which includes poor management of diabetes, antibiotic resistance and impaired blood circulation. Non healing DFU has become a major healthcare burden, cause for longer hospital admissions, expensive treatment cost to developing countries and causing negative impact on patients' quality of life (Kee, Nair, & Yuen, 2019; Yimam, Hailu, Murugan, & Gebretensaye, 2021). It has been reported that the lifetime risk for a diabetic patient to have DFU is estimated at 25% and about 84% of lower limb amputations were preceded by diabetic foot complications. Body of evidences has indicated diabetic-foot-based amputations were 10 to 20 times higher as compared to non-diabetics (Acar & Kacıra, 2017; Atosona & Larbie, 2019). A statement released by the International Diabetes Federation (2019) mentioned that one limb is lost due to DFU somewhere in the world which clearly indicate the seriousness of diabetes-based-foot complications across the globe (International Diabetes Federation, 2019; Saeedi et al., 2019). Managing and improving wound healing outcomes of DFU has become quite a challenge for clinicians as the healing process becomes prolonged and causes unnecessary socio-economic burden to the patients. In order for chronic wound to heal, the wound needs to progress into a normal healing phase which includes haemostasis, inflammation, proliferation and maturation. However, majority of chronic wounds gets stuck in the inflammation stage and does not move into proliferation phase (Guo & DiPietro, 2010). Therefore, inflammation phase is prolonged and causes much damage to the cells in the wound bed. Research findings has shown that oxidative stress which is created by excess in reactive oxygen species (ROS) or free radicals is one the predominant factor which contributes to the chronicity of the wound besides infection, biofilm and antibiotic resistance (Dupont et al., 2020). In recent times, chronic wound management has taken big leaps to stimulate conducive environment in the wound bed in order to improve healing outcomes and reduce foot complications. As the modern wound care evolves drastically, resurgence of traditional remedies to overcome the challenges in chronic wound management has been quite prominent.

One of the traditional remedies which has been highly evaluated and clinically proven to stimulate wound healing is honey (Jull et al., 2015; Meo, Al-Asiri, Mahesar, & Ansari, 2017). Previous findings had shown that honey's antibacterial, anti-inflammation, and antioxidant therapeutic properties, low pH and high

osmolarity were the main associated factors related to improved wound healing in burns and other type of wounds (Alvarez-Suarez, Gasparrini, Forbes-Hernández, Mazzoni, & Giampieri, 2014; Mandal & Mandal, 2011; McLoone, Oluwadun, Warnock, & Fyfe, 2016). Honey has been produced worldwide but not all honey is same as the pharmacological properties is very much dependent on where it is harvested and which plant the bees visit, and the environment the honey is produced in (Gill, Poojar, Bairy, & Praveen, 2019; Shirlaw et al., 2020). There are various types of honey across the globe but the most common, commercially formulated for topical wound dressing is manuka honey or Leptospermum honey which is a honey bee *Apis spp*. These sting bees pollinate the Leptospermum tree found mainly in New Zealand. They are monofloral, produce abundance of honey, prominent in economically-based beekeeping and some are even found in Malaysia like Tualang honey. Manuka honey contains high concentration of methylglyoxal (MGO), derived from the pollen and nectar collected from Leptospermum tree (found in New Zealand) (Vyhlídalová, Kozáková, & Zeleníková, 2018). With these therapeutic properties, manuka honey has become a potential topical wound dressing in the form of gel and impregnated gauze. Medical-grade manuka honey was formulated to reduce oxidative stress, biofilm, antibiotic resistance and enhance chronic wound healing (Ooi, Yaacob, Rajab, Shahar, & Sharif, 2021; Angioi, Morrin, & White, 2021). On the contrary sting-less bees is poly-floral and the stigma that they produce less honey as compared to sting bees which made it less popular and least researched (Al-Hatamleh et al., 2020). Stingless bees have been identified as a small non-aggressive bee from the species of *Trigona* or *Meliponine* and were identified with different names in different regions (Aina et al., 2018). For example, it is known as “damar” in India and “lukut” in Philipines. In Malaysia, the local stingless bees are called kelulut bees (Zainol et al., 2013). According to the Malaysian Agriculture Research and Development Institut (MARDI) (2014), there are about 500 species of stingless bee in the world and 35 species identified in Malaysia. However, four local kelulut bee species have been successfully identified and included in beekeeping in the local context. Yaacob, Rajab, Shahar, and Sharif (2017) mentioned the four known species of stingless bees in Malaysia are *Trigona itama*, *Trigona apicalis*, *Trigona carnifrons* and *Trigona thoracica*. Their size ranges from 2 - 5 mm and true to their name, it has no stinger. According to Kek, Chin, Yusof, Tan, and Chua, (2014), the kelulut bees are highly sociable, with one queen living together. The kelulut industry gained wide attention in recent times as this honey was listed as a Malaysian superfood by MARDI in 2016 (Ismail & Ismail, 2018). The potential for kelulut beekeeping in Malaysia started to gain momentum since the handling of these bees were found to be more user-friendly’ as compared to honeybee that has sting property (Khalil, Alam, Moniruzzaman, Sulaiman, & Gan, 2011). Consequently, exploration into rearing of kelulut bees as an economic proposition had increased the production of kelulut honey in the local market. Few reports pointed to the fact that kelulut bees in Malaysia produced more honey than honeybees from Australia and New Zealand (Ooi et al., 2021; Yaacob et al., 2017).This revelation had denounced the earlier claim that the quantity of honey produced by stingless was lower than sting bees. As the quantity of kelulut honey increased, research into its nutritional values began and analytics showed the nutritional value of kelulut honey was similar to honeybees. Therefore, kelulut honey was introduced for consumption as superfood for maintaining good health (Brown, O’Brien, Georges, & Suepaul, 2020; Rashid et al., 2019; Ulasan et al., 2020; Vit, Roubik, & Pedro, 2012). Nevertheless, research on kelulut honey for wound healing evaluation is certainly lacking (Abdul Karim & Anum, 2019) despite reports demonstrating higher antioxidant properties in kelulut honey as compared to manuka honey(Brown et al., 2020; Rao, Krishnan, Salleh, & Gan, 2016; Yaacob et al., 2017). Other pointers showing more reasons for kelulut honey to be considered for therapeutic use include lower minimum inhibition concentration (MIC) value

(an indicator for antibacterial properties of honey) in kelulut honey than manuka honey, propolis, and artificial honey. Similar to manuka honey, the anti-bacterial properties demonstrated by kelulut honey could be used in managing the ever growing antibiotic-resistance in the treatment of chronic wounds (Andualem, 2013; Ewnetu, Lemma, & Birhane, 2013; Selvaraju, Vikram, Soon, Krishnan, & Mohammed, 2019). The higher antioxidant properties in kelulut honey will be the added advantage as oxidative stress which is responsible for inflammation of cells and infection could be reduced and improve healing outcomes in chronic wounds such as DFU. It is highly anticipated that clinical outcomes may differ between the manuka and kelulut honey in wound healing based on the therapeutic properties. However, comparison studies between manuka honey and kelulut honey is yet to be forthcoming. Hence, the undertaken study isto fill the gap of knowledge for kelulut honey as a potential topical wound dressing in comparison to manuka honey for the treatment of non-healing DFU in clinical setting.

Hypotheses

The hypotheses for the undertaken study include 1) treating DFU with kelulut honey would demonstrate higher percentage of wound size reduction 2) treating DFU with kelulut honey would demonstrate faster wound size reduction 3) healing rate with kelulut honey could be comparable or potentially higher than medical-grade manuka honey in the treatment of DFU.

Study objectives

The main objective of the study is to evaluate the efficacy of kelulut honey and medical-grade manuka honey in the treatment of DFUs based on the healing rate by measuring the wound size reduction from baseline to day 7 and day 14.

The secondary objective is to assess if there is any relationship between participants' socio-demography (age, gender), clinical characteristics (duration of diabetes, HbA1c), wound characteristics (grade, size, site, duration of ulcer) and the healing rate of DFU.

Method

Research Design

The study will be a single-blinded experimental study conducted in a tertiary teaching hospital, University Malaya Medical Centre (UMMC) on participants with DFU.

Study Setting

UMMC is selected because the setting represents the cross-segmentation of the Malaysian society which provides the descriptive healthcare environment where the applicability of the findings may be possible, ethics oversight, and availability of appropriate resources. Furthermore, UMMC is located on the border of Kuala Lumpur-Selangor and is a referral site for peripheral health clinics and district hospitals.

Sample Size

To fulfill the aim of the study, the calculation of sample size is based on producing a higher probability of achieving the targeted outcome, at least a minimum 20 % reduction of size in the DFUs. The calculation will be based on previous similar interventional studies with minimum detectable size in the treatment of DFUs (Marimuthu & Makhtar, 2020; Opletalová et al., 2012) and interventional study with manuka honey (Astrada et al., 2019; B. Biglari et al., 2012; A. Jull et al., 2008; Xu Tian et al., 2014). The calculation showed a minimum of 55 samples required to determine the statistical significance in the outcome of the undertaken study in each group (with a standard deviation of 37.5%, error estimate 5% with 80 % probability of detecting changes in the size of ulcer). In consideration of the possible 10% dropout, 60 participants will be recruited for the study.

Study Population

The participants will consist of adult diabetic patients with sloughy foot ulcers presented to the Outpatient Orthopaedic Clinic in UMMC. The selection of participants for the study is based on

inclusion and exclusion criteria. Most importantly, signed consent from either patient or family member will be collected before commencement of the study. Participants who have consented to the study will undergo debridement with maggot therapy in Phase 1 and honey dressing in Phase 2. The participants will be randomized into 2 groups (intervention Group 1 treated with kelulut honey & control Group 2 treated with medical-grade manuka honey).

Recruitment Criteria

Inclusion and exclusion criteria

The inclusion criteria include adult diabetics with sloughy foot ulcer, not requiring urgent debridement, not having sepsis or life-threatening infections, non-ischemic, no profuse bleeding, and decision to amputate could be deferred. On the contrary, the main exclusion criteria will be patients who need urgent debridement, contracted life-threatening infection like sepsis and ischemic pain.

Randomization

Participants will be randomized using a simple random sampling technique. Group allocation of 1 & 2 will be written on paper and folded 4 times and will be enclosed in an envelope. As the participants complete recruitment procedure, they will be asked to select the envelope. The nurse in the study team will open the envelope and assign the participants accordingly where participants who selects No 1 will be recruited for Group 1and No 2 in Group 2. The participant will be blinded on the type of honey applied to the wound during the study period.

Wound Assessment

Wound characteristics of DFU such as grade, site, duration of ulcer, and the size of ulcer will be included in the baseline documentation as they may be associated risk factors for wound healing as shown in previous studies (Smith-Strøm et al., 2017; Ugwu et al., 2019). Based on the guidelines recommended by the International Working Group on the Diabetic Foot (IWGDF), validated SINBAD (Site, Ischemia, Neuropathy, Bacterial infection, Depth) will be used for wound grading.

Wound Measurement

NDKare™ application will be used for wound measurement in the study as it allows estimation of length, width, depth, necrosis, slough, and granulation tissue. NDKare™ is a validated system, FDA approved, CE marked for documentation of wound progress and to formulate strategies to expedite healing of chronic wounds. Special stickers will be placed beside the wound before the wound images are captured using the application as reference for the wound area measurements. Wound images and measurements will be displayed on the smartphone each time the wound image is captured and uploaded instantly. The documentation will be stored in the NDKare™ app database and can be retrieved by the principal investigator securely. This app will be used to measure the size of ulcers in both intervention and control groups at baseline, day 7, and day 14.

Table 1 illustrates the overall schedule for recruitment, maggot debridement, intervention and assessment

	TIMEFRAME			
Activities/Time	Initial visit	1st follow up	2nd follow up	Post follow up
PHASE 1:RECRUITMENT				
Initial Participant Screening	x			
Selection(Inclusion & Exclusion Criteria)	x			
Informed Consent	x			
PHASE 2:MAGGOT DEBRIDEMENT				
Maggot Application	x			
Follow up	x			
1st Cycle	x			
2nd Cycle	x			
PHASE 3:INTERVENTION				
Participant Randomization	x			
Baseline Wound Measurement	x			
Kelulut Honey Dressing		←	→	
Manuka Honey Dressing		←	→	
Data Collection				
1st Measurement		x		
2nd Measurement			x	
Wound Photography		←	→	
PHASE 4:ASSESSMENT				
Wound Analysis				x
Socio-Demographic Characteristics				x
Clinical Characteristics				x
Adverse Events		x	x	x

Data Collection

The study protocol will be explained to all the participants prior to obtaining their consent. Informed consent forms will be either signed by the participant or the relative. The consented participants will be each given a specific patient identifier number, 001 till 060. All the participants' personal & clinical data will be documented and kept in an excel format on the computer. Confidentially of the participants data are strictly adopted. The wound measurements will be stored in a computer with a password encoded and stored securely. Hard copies will be kept securely at the Orthopedic Dept under the purview of the principal investigator. The measurement of wounds will begin at baseline in Phase 2 where the application of honey will commence and periodical measurement will continue on day 7 and day 14, with dressing change will be performed every other day. All the participants will be treated in the dressing room by the same nurses involved in the study. To retain and support the patient at home, participants will be monitored by the nurses and investigator. The principal investigator will ensure the recruitment of participants follows the selection criteria and follow up for dressing change and measurement of wounds are performed according to the application protocol.

Phase 1: Debridement

Maggot debridement therapy

The recruited participants will undergo maggot debridement therapy (MDT) as wound debridement in chronic wounds is the science behind wound bed optimization to create an optimal healing environment (Bazaliński, Kózka, Karnas, & Więch, 2019). Removal of non-viable tissue is crucial since it could become a "nutrient source" for bacteria to multiply, possibly create a biofilm (Lauerman et al., 2018), act as a barrier for topical dressing application to penetrate and produce their potency (Chiwanga & Njelekela, 2015; Everett & Mathioudakis, 2018; Kavitha et al., 2014; Lebrun, Tomic-Canic, & Kirsner, 2010). Numerous clinical evidences has indicated sterile maggots of *Lucilia* spp as a potent

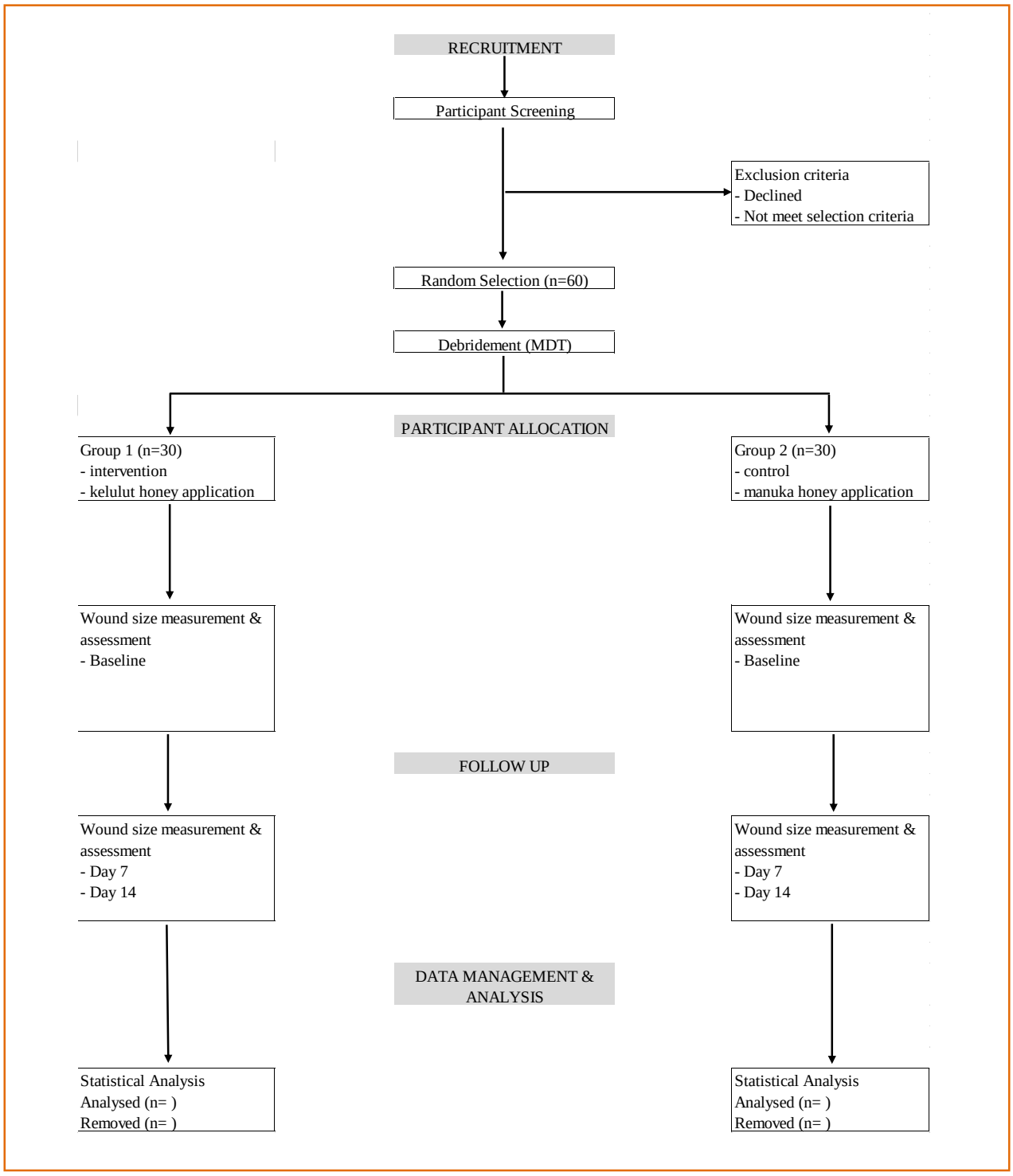
debridement tool to disinfect, increase granulation and enhance healing in DFU (Bazaliński, Kózka, Karnas, & Więch, 2019; Jordan, Khiyani, Bowers, Lukaszczyk, & Stawicki, 2018; Nair, Wasi Ahmad, Teh, Lee, & Chong, 2020). MDT using *Lucilia cuprina* in the local context had shown significant better outcomes in debriding sloughy wounds as compared to non-surgical methods (Marimuthu & Makhtar, 2020) and comparable to surgical methods (Paul et al., 2009). Since sterile maggots for MDT is readily available and majority of healthcare settings utilize it for DFU, MDT was selected to be used in the study.

For the study, sterile maggots will be ordered from the Institute of Medical Research, Kuala Lumpur (IMR) where the colony of *Lucilia cuprina* is kept in a controlled environment. Sterilization of the eggs is done at IMR using a patented method and the larva hatched from the eggs are collected with a special scoop into a sterile vial. Each vial can accommodate 100 – 250 sterile maggots (Yeong et al., 2011). The vials will be packed in a cooler box together with sterile plastic forceps and delivered to the hospital for application. Once the maggots reach the hospital, the patient's wound will be cleaned with normal saline aseptically. The maggots will be applied to the wound soonest possible since the timeline for the life of the maggots is 24 hours. Only normal saline is to be poured into the vial to dispense the maggots, collected with sterile forceps and transferred onto sterile gauze. The sterile gauze with maggots will be placed on the wound. A cage dressing using sterile gauze will be applied around the wound area and sealed with hyperfix to prevent maggots from crawling out. The dressing will be enforced with secondary dressing using sterile gamgee or thick gauze and bandaged. Patients will be advised not to wet the foot and minimize walking and if possible, rest in bed. The dressing is to be opened after 72 hours and maggots will be removed and may be reapplied if the wound does not achieve complete debridement. Based on the previous clinical data, it may take 1-2 cycle of 100-200 maggots to achieve complete debridement within 3-6 days. Once the wounds achieve complete debridement, the study population will proceed through Phase 2.

Phase 2: Intervention

Upon completing Phase 1, the study population will move on to phase 2 whereby intervention with respective honey dressing based on group allocation will be followed through. Medical-grade manuka honey is used as the control because this honey is used as the honey dressing in the treatment of DFUs locally in healthcare settings. The kelulut honey and medical-grade manuka honey dressing will be labelled as 1 & 2 and kept in amber bottle in the Orthopaedic Treatment Room. Prior to the honey application, the wounds will be measured to determine the size at baseline. All wounds in both groups will be cleansed with normal saline aseptically As mentioned earlier, Group 1 will be treated with kelulut honey & Group 2 with medical-grade manuka honey. The application protocol of medical-grade manuka honey onto the surface of DFU will be similar to kelulut honey where the required amount of honey will be evenly spread on the surface of the wound. Sterile gauze will be placed on the wound as primary dressing, will be covered with gamgee as secondary dressing and bandaged. Application and dressing change will be done by the nurse in the study team. Wound dressing with kelulut and medical-grade manuka honey will be continued and changed every other day. The size of the ulcers will be continuously measured on day 7 and day 14 to determine the healing rate. All participants will be required to come for follow up every other day for dressing change. The participants will be reminded on the importance of keeping the dressing intact and not removing the dressing until the scheduled visit which is every other day. The participants will be advised as well to inform the researcher (contact number stated in the patient information sheet) if they are experiencing pain or bleeding or any other symptoms related to the wound. Participants may be given analgesics if mild pain is observed or even will be asked to return to the clinic to remove the dressing if symptoms persisted. These symptoms will be documented as possible adverse effects and if the participant decides to quit the study, he or she will be removed from the study and will be referred back to the Orthopaedic team for further action.

Figure 1 Data Collection Summary



Data Analysis

The proposed statistical analysis will be done using IBM Statistical Package for Social Sciences Version 28. Baseline characteristics of the participants and characteristics will be collected and types of variables differentiated into dependent and independent variables. Independent variables will include participants' socio-demography, clinical characteristics, and wound characteristics at baseline whereas dependent variables consist of size measurement. Descriptive statistics and normality testing will be performed. To compare the efficacy of kelulut honey and medical-grade manuka honey in the treatment of DFUs, repeated measures Rm-ANCOVA will be used to determine the mean size reduction at day 7 and day 14 as compared to baseline measurement in both groups. Independent t-test will be used to evaluate if there exist significant differences in the healing rate between the 2 groups. The differences between both groups will be tested at 95% confidence level, 5% significance level ($p<0.05$).Statistical testing will be also conducted to establish the relationship between patient socio-demography, wound characteristics, and outcome of the study (size of ulcer). In the context of continuous data, Pearson correlation testing will be performed to evaluate the relationship between age, HbA1c, and size of the ulcer. Based on categorical data, Spearman's correlation testing will be used to determine the relationship between the duration of diabetes, duration of ulcer, and size of ulcer in both groups. Thus, sub-analysis using Mann-Whitney U will be performed to determine the relationship between gender, site of ulcer, wound characteristics grading, and size of ulcer. A significance level of $p < 0.05$ at 95% confidence will be used to determine the statistical significance between the variables and the outcome of the undertaken study. In addition, analysis will also be performed to assess if there is a relationship between participants' comorbidities and healing rate.

Outcome

The primary outcome of the study will be based on the changes in the size of wounds from baseline to day 7 and day 14. Measurement of wounds on day 7 and 14 will show the efficacy of kelulut honey at different endpoints in terms of granulation and healing rate. The percentage of wound size reduction will indicate the differences in healing rate between the two groups.

Discussion

In the historical perspective, it was postulated that conventional methods using saline moistened gauze predominated as a standard dressing in the majority of healthcare settings before 1980s (Vowden & Vowden, 2014). In this era, wound dressings tend to play an important role in chronic wound management as it forms a base for contact with the wound and expedite healing. Selection of appropriate topical wound dressing is very much aimed at inhibiting bacterial activity, promote angiogenesis, enhance epidermal migration, allow gas exchange between the wound & environment, maintain tissue temperature, non-adherent to wound, effective exudate management, debride, safe and non-toxic (Dhivya, Padma, & Santhini, 2015). Latest findings stressed that more importantly, the topical wound dressing should be wound-specific and provide moist wound healing (Kordestani, 2019). Undoubtedly, wound dressing had gone through evolutionary paradigm shift from a mere usage of gauze and cotton to advance modern dressing that focuses on moist wound healing as wounds turn chronic and complicated (Kiliçoglu, Demirel, & Aktas, 2018). At one point as the incidences of non-healing ulcers increased in the mid 90's, advanced dressing such as silver alginate dressing, collagen dressing, hyaluronic acid, hydrogels, synthetic foam dressing, vapor-permeable adhesive films, povidone gel, hydrocolloids, and polyurethane foams were brought to the forefront of chronic wound treatment (Dumville et al., 2017; Kiliçoglu et al., 2018). However, very few have prospective data to support their efficacy in enhancing wound

healing and some were reported to be ineffective towards inhibiting bacterial growth, managing exudates and ultimately were not able to enhance granulation and shorten healing time (Frykberg & Banks, 2015; Mavrogenis et al., 2018; Tsourdi, Barthel, Rietzsch, Reichel, & Bornstein, 2013). Topical wound dressing containing antimicrobial properties became more sought after in the modern wound care management as antibiotic resistance leading to severe foot complications increases(Andrews, Houdek, & Kiemele, 2015; Dumville et al., 2017; Sarabahi, 2012; Vowden & Vowden, 2014). It is paramount for new dressing to be explored to weigh down the impact of foot complications. Topical wound dressing using medical-grade manuka honey has been long accepted across the globe as one of the alternative modern dressing as it has been highly researched, abundant and USFDA approved for wounds in 2015. Stingless bee honey like the local kelulut honey clearly lacks introduction in the field of chronic wound management as it has not progressed into in vitro clinical wound evaluations. As mentioned, the outcome of the study could open up window of probability for kelulut honey to be used for managing infection, antibiotic resistance and promote healing; which is very much similar to manuka honey. Inadvertantly, the finding could suggest superior healing rate with kelulut honey due to its higher antioxidant properties which has been demonstrated in many previous studies (Abd Jalil, Kasmuri, & Hadi, 2017; Lima, Brito, & da Cruz Nizer, 2020). Topical dressing using kelulut honey could be a potential addition to modern wound care as it could reduce oxidative stress which is a contributory factor for infection, inflammation and delayed wound healing (Al-Hatamleh et al., 2020). Furthermore, kelulut honey dressing may bring added healing advantage as it has been indicated to have an additional effect on angiogenesis and oxygen circulation based on the clinical data in previous findings (Chong, Chin, Yusof, & Fakurazi, 2021). Hence, it may be possible to procast that kelulut honey may produce comparable or superior healing rate than medical-grade manuka honey in the treatment of DFU in the study. Thus, kelulut honey could be considered as an evidence-based topical dressing in chronic wound management if the outcome concurs with the hypothesis. On another note, this will be one of the first scientific exploration to combine MDT and honey dressing for DFU at the point of time. Thus, the window of usage for MDT could be widened as a complete wound treatment package for the healing of DFU with honey dressing to complete healing process given the body of evidences and clinical efficacies. Consequently, this study will widen the window of usage for kelulut honey to produce therapeutic efficacies in chronic wound healing. As the infection rate in non-healing DFU reaches as high as 41% in the latest study conducted at a local public healthcare setting as mentioned by Kee et al (2019), utilizing kelulut honey in topical dressing could reduce infection, inflammation, antibiotic resistance, oxidative stress, enhance healing and wound closure in DFU which could possibly reduce the number of lower limb amputations. Future randomized controlled studies with kelulut honey should be performed on a larger scale to produce level 1 evidence to establish kelulut honey as a potent topical dressing for chronic wound management.

Ethical Consideration & Participants Consent

This study was approved by Kulliyah of Nursing, and registered with National Medical Research Registrar (NMRR).Good Clinical Practice Certificate (GCP) was also submitted as a pre-requisite for the application approval. Participants were required to give written consent and sign the informed consent form, prior to participation in the study. The patient consent form and patient information sheet were approved by the UMMC Research Ethics Committee.

References

Abd Jalil, M. A., Kasmuri, A. R., & Hadi, H. (2017). Stingless Bee Honey, the Natural Wound Healer: A Review. *Skin Pharmacology and Physiology*, *30*(2), 66–75. https://doi.org/10.1159/000458416

Abdul Karim, N., & Anum, Y. M. (2019). *Antioxidant Properties of Stingless Bee Honey and Its Effect on the Viability of Lymphoblastoid Cell Line*. *14*(1), 91–105. https://doi.org/10.17576/MH.2019.1401.08

Acar, E., & Kacıra, B. K. (2017). Predictors of Lower Extremity Amputation and Reamputation Associated With the Diabetic Foot. *Journal of Foot and Ankle Surgery*, *56*(6). https://doi.org/10.1053/j.jfas.2017.06.004

AG, P., NW, A., HL, L., AM, A., Saranum, M., AS, N., & Osman, Z. (2009). Maggot debridement therapy with Lucilia cuprina: a comparison with conventional debridement in diabetic foot ulcers. *International Wound Journal*.

Aina, F., Amin, Z., Sabri, S., Mohammad, S. M., Ismail, M., Chan, K. W., … Rasul, A. (2018). *Therapeutic Properties of Stingless Bee Honey in Comparison with European Bee Honey*.

Al-Hatamleh, M. A. I., Boer, J. C., Wilson, K. L., Plebanski, M., Mohamud, R., & Mustafa, M. Z. (2020). Antioxidant-based medicinal properties of stingless bee products: Recent progress and future directions. *Biomolecules*, *10*(6), 1–28.

Al-Rubeaan, K., Al Derwish, M., Ouizi, S., Youssef, A. M., Subhani, S. N., Ibrahim, H. M., & Alamri, B. N. (2015). Diabetic foot complications and their risk factors from a large retrospective cohort study. *PloS One*, *10*(5), e0124446.

Alvarez-Suarez, J. M., Gasparrini, M., Forbes-Hernández, T. Y., Mazzoni, L., & Giampieri, F. (2014). *The Composition and Biological Activity of Honey: A Focus on Manuka Honey*. *3*, 420–432.

Andrews, K. L., Houdek, M. T., & Kiemele, L. J. (2015). Wound management of chronic diabetic foot ulcers: From the basics to regenerative medicine. *Prosthetics and Orthotics International*, Vol. 39, pp. 29–39.

Andualem, B. (2013). Combined antibacterial activity of stingless bee (Apis mellipodae) honey and garlic (Allium sativum) extracts against standard and clinical pathogenic bacteria. *Asian Pacific Journal of Tropical Biomedicine*, *3*(9), 725–731.

Atosona, A., & Larbie, C. (2019). *Prevalence and Determinants of Diabetic Foot Ulcers and Lower Extremity Amputations in Three Selected Tertiary Hospitals in Ghana*.

Bazaliński, D., Kózka, M., Karnas, M., & Więch, P. (2019a). Effectiveness of Chronic Wound Debridement with the Use of Larvae of Lucilia Sericata. *Journal of Clinical Medicine*, *8*(11), 1845.

Bazaliński, D., Kózka, M., Karnas, M., & Więch, P. (2019b). Effectiveness of Chronic Wound Debridement with the Use of Larvae of Lucilia Sericata. *Journal of Clinical Medicine*, *8*(11), 1845.

Brown, E., O'Brien, M., Georges, K., & Suepaul, S. (2020). Physical characteristics and antimicrobial properties of Apis mellifera, Frieseomelitta nigra and Melipona favosa bee honeys from apiaries in Trinidad and Tobago. *BMC Complementary Medicine and Therapies*, *20*(1), 85.

Chiwanga, F. S., & Njelekela, M. A. (2015). Diabetic foot: Prevalence, knowledge, and foot self-care practices among diabetic patients in Dar es Salaam, Tanzania - a cross-sectional study. *Journal of Foot and Ankle Research*, *8*(1).

Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W., & Malanda, B. (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research and Clinical Practice*, *138*.

Chong, K. Y., Chin, N. L., Yusof, Y. A., & Fakurazi, S. (2021). Anti-inflammatory activity of raw and processed stingless bee honey. *Food Research*, *5*.

Chun, D., Kim, S., Kim, J., Yang, H.-J., Kim, J. H., Cho, J., … Won, S. H. (2019). Epidemiology and Burden of Diabetic Foot Ulcer and Peripheral Arterial Disease in Korea. *Journal of Clinical Medicine*, *8*(5), 748.

Dhivya, S., Padma, V. V., & Santhini, E. (2015, December 1). Wound dressings - A review. *BioMedicine (Netherlands)*, Vol. 5, pp. 24–28.

Dumville, J. C., Lipsky, B. A., Hoey, C., Cruciani, M., Fiscon, M., & Xia, J. (2017, June 14). Topical antimicrobial agents for treating foot ulcers in people with diabetes. *Cochrane Database of Systematic Reviews*, Vol. 2017.

Dupont, C., Brescó, M. S., Martins-Green, M., Kim, J. H., Ruegger, P. R., Lebig, E. G., … Borneman, J. (2020). High Levels of Oxidative Stress Create a Microenvironment That Significantly Decreases the Diversity of the Microbiota in Diabetic Chronic Wounds and Promotes Biofilm Formation. *Frontiers in Cellular and Infection Microbiology | Www.Frontiersin.Org*, *1*, 259.

Everett, E., & Mathioudakis, N. (2018). Update on management of diabetic foot ulcers. *Annals of the New York Academy of Sciences*, Vol. 1411.

Ewnetu, Y., Lemma, W., & Birhane, N. (2013). Antibacterial effects of Apis mellifera and stingless bees honeys on susceptible and resistant strains of Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae in Gondar, Northwest Ethiopia. *BMC Complementary and Alternative Medicine*, *13*.

Frykberg, R. G., & Banks, J. (2015). Challenges in the Treatment of Chronic Wounds. *Advances in Wound Care*, *4*(9), 560–582.

Gill, R., Poojar, B., Bairy, L. K., & Praveen, K. S. E. (2019). Comparative evaluation of wound healing potential of manuka and acacia honey in diabetic and nondiabetic rats. *Journal of Pharmacy and Bioallied Sciences*, *11*(2), 116–126.

Gubara Musa, H., & E. Ahmed, M. (2012). Associated risk factors and management of chronic diabetic foot ulcers exceeding 6 months' duration. *Diabetic Foot & Ankle*, *3*(1), 18980.

Guo, S., & DiPietro, L. A. (2010). Critical review in oral biology & medicine: Factors affecting wound healing. *Journal of Dental Research*, *89*(3), 219–229.

Hinchliffe, R. J., Brownrigg, J. R. W., Apelqvist, J., Boyko, E. J., Fitridge, R., Mills, J. L., … Schaper, N. C. (2016). IWGDF guidance on the diagnosis, prognosis and management of peripheral artery disease in patients with foot ulcers in diabetes. *Diabetes/Metabolism Research and Reviews*, *32*.

Ismail, M. M., & Ismail, W. I. W. (2018). Development of stingless beekeeping projects in Malaysia. *E3S Web of Conferences*, *52*.

Jordan, A., Khiyani, N., Bowers, S., Lukaszczyk, J., & Stawicki, S. (2018). Maggot debridement therapy: A practical review. *International Journal of Academic Medicine*, *4*(1), 21.

Jull, A. B., Cullum, N., Dumville, J. C., Westby, M. J., Deshpande, S., & Walker, N. (2015, March 6). Honey as a topical treatment for wounds. *Cochrane Database of Systematic Reviews*, Vol. 2015.

Kavitha, K. V., Tiwari, S., Purandare, V. B., Khedkar, S., Bhosale, S. S., & Unnikrishnan, A. G. (2014). Choice of wound care in diabetic foot ulcer: A practical approach. *World Journal of Diabetes*, *5*(4), 546–556.

Kee, K. K., Nair, H. K. R., & Yuen, N. P. (2019). Risk factor analysis on the healing time and infection rate of diabetic foot ulcers in a referral wound care clinic. *Journal of Wound Care*, *28*(Sup1), S4–S13.

Kek, S. P., Chin, N. L., Yusof, Y. A., Tan, S. W., & Chua, L. S. (2014). Total Phenolic Contents and Colour Intensity of Malaysian Honeys from the Apis spp. and Trigona spp. Bees. *Agriculture and Agricultural Science Procedia*, *2*, 150–155.

Khalil, M. I., Alam, N., Moniruzzaman, M., Sulaiman, S. A., & Gan, S. H. (2011). Phenolic Acid Composition and Antioxidant Properties of Malaysian Honeys. *Journal of Food Science*, *76*(6).

Kiliçoglu, Ö. I., Demirel, M., & Aktas, S. (2018). New trends in the orthopaedic management of diabetic foot. *EFORT Open Reviews*.

Kordestani, S. S. (2019). Wound Care Management. In *Atlas of Wound Healing* (pp. 31–47).

M., & Henry, S. (2018). *Efficacy of Wound Coverage Techniques in Extremity Necrotizing Soft Tissue Infections*. *The American Surgeon*, *84*(11), 1790–1795.

Lebrun, E., Tomic-Canic, M., & Kirsner, R. S. (2010). The role of surgical debridement in healing of diabetic foot ulcers. *Wound Repair and Regeneration*, *18*(5), 433–438.

Lima, W. G., Brito, J. C. M., & da Cruz Nizer, W. S. (2020). Bee products as a source of promising therapeutic and chemoprophylaxis strategies against COVID-19 (SARS-CoV-2). *Phytotherapy Research*.

Mandal, M. D., & Mandal, S. (2011). Honey: Its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, *1*(2), 154–160.

Marimuthu, P., & Makhtar, A. (n.d.). *Effectiveness of Maggot Debridement Therapy (Luciliacuprina) In the Treatment of Diabetic Foot Ulcer*. *9*(2), 50–56.

Maringgal, B., Hashim, N., Syafinaz, I., Amin Tawakkal, M., Tengku, M., Mohamed, M., … Shukor, A. (2019). SCIENCE & TECHNOLOGY Phytochemical Compositions and Antioxidant Activities of Malaysian Stingless Bee Honey. *Pertanika J. Sci. & Technol*, *27*(S1), 15–28.

Mavrogenis, A. F., Megaloikonomos, P. D., Antoniadou, T., Igoumenou, V. G., Panagopoulos, G. N., Dimopoulos, L., … Lazaris, A. (2018, September 1). Current concepts for the evaluation and management of diabetic foot ulcers. *EFORT Open Reviews*, Vol. 3, pp. 513–525.

McLoone, P., Oluwadun, A., Warnock, M., & Fyfe, L. (2016). Honey: A Therapeutic Agent for Disorders of the Skin. *Central Asian Journal of Global Health*, *5*(1).

Meo, S. A., Al-Asiri, S. A., Mahesar, A. L., & Ansari, M. J. (2017, July 1). Role of honey in modern medicine. *Saudi Journal of Biological Sciences*, Vol. 24, pp. 975–978.

Monteiro-Soares, M., Martins-Mendes, D., Vaz-Carneiro, A., Sampaio, S., & Dinis-Ribeiro, M. (2014). Classification systems for lower extremity amputation prediction in subjects with active diabetic foot ulcer: a systematic review and meta-analysis. *Diabetes/Metabolism Research and Reviews*, *30*(7), 610–622.

Nair, H. K. R., Wasi Ahmad, N., Teh, C. H., Lee, H. L., & Chong, S. S. Y. (2020). Maggot Debridement Therapy in Malaysia. *International Journal of Lower Extremity Wounds*.

Noor, S., Khan, R. U., & Ahmad, J. (2017). Understanding Diabetic Foot Infection and its Management. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, Vol. 11.

Omar, S., Farhana Mat-kamir, N., & Sanny, M. (2019). ANTIBACTERIAL ACTIVITY OF MALAYSIAN PRODUCED STINGLESS-BEE HONEY ON WOUND PATHOGENS. In *Journal of Sustainability Science and Management* (Vol. 14).

Ooi, T. C., Yaacob, M., Rajab, N. F., Shahar, S., & Sharif, R. (2021). The stingless bee honey protects against hydrogen peroxide-induced oxidative damage and lipopolysaccharide-induced inflammation in vitro. *Saudi Journal of Biological Sciences*, *28*(5).

Paul, A. G., Ahmad, N. W., Lee, H., Ariff, A. M., Saranum, M., Naicker, A. S., & Osman, Z. (2009). Maggot debridement therapy with Lucilia cuprina: A comparison with conventional debridement in diabetic foot ulcers. *International Wound Journal*, *6*(1), 39–46.

Rao, P. V., Krishnan, K. T., Salleh, N., & Gan, S. H. (2016, September 1). Biological and therapeutic effects of honey produced by honey bees and stingless bees: A comparative review. *Revista Brasileira de Farmacognosia*, Vol. 26, pp. 657–664.

Rashid, M. R., Nor Aripin, K. N., Syed Mohideen, F. B., Baharom, N., Omar, K., Md Taujuddin, N. M. S., … Addnan, F. H. (2019). The Effect of Kelulut Honey on Fasting Blood Glucose and Metabolic Parameters in Patients with Impaired Fasting Glucose. *Journal of Nutrition and Metabolism*, *2019*.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., … Williams, R. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, *157*.

Sarabahi, S. (2012, May). Recent advances in topical wound care. *Indian Journal of Plastic Surgery*, Vol. 45, pp. 379–387.

Selvaraju, K., Vikram, P., Soon, J. M., Krishnan, K. T., & Mohammed, A. (2019). Melissopalynological, physicochemical and antioxidant properties of honey from West Coast of Malaysia. *Journal of Food Science and Technology*, *56*(5), 2508–2521.

Shirlaw, O., Billah, Z., Attar, B., Hughes, L., Qasaymeh, R. M., Seidel, V., & Efthimiou, G. (2020). Antibiofilm activity of heather and manuka honeys and antivirulence potential of some of their constituents on the DsbA1 enzyme of pseudomonas aeruginosa. *Antibiotics*, *9*(12), 1–12.

Smith-Strøm, H., Iversen, M. M., Igland, J., Østbye, T., Graue, M., Skeie, S., … Rokne, B. (2017). Severity and duration of diabetic foot ulcer (DFU) before seeking care as predictors of healing time: A retrospective cohort study. *PLoS ONE*, *12*(5).

Syafril, S. (2018). Pathophysiology diabetic foot ulcer. *IOP Conference Series: Earth and Environmental Science*, *125*(1).

Tsourdi, E., Barthel, A., Rietzsch, H., Reichel, A., & Bornstein, S. R. (2013). Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. *BioMed Research International*.

Ugwu, E., Adeleye, O., Gezawa, I., Okpe, I., Enamino, M., & Ezeani, I. (2019). Predictors of lower extremity amputation in patients with diabetic foot ulcer: findings from MEDFUN, a multi-center observational study. *Journal of Foot and Ankle Research*, *12*, 34.

Ulasan, S., Bakteria Dengan Kelulut, P., Syazwan Ngalimat, M., Noor, R., Raja, Z., Rahman, A., … Sabri, S. (2020). A Review on the Association of Bacteria with Stingless Bees. *Sains Malaysiana*, *49*(8), 1853–1863. Van Dieren, S., Beulens, J. W. J., Van Der Schouw, Y. T., Grobbee, D. E., & Neal, B. (2010, May). The global burden of diabetes and its complications: An emerging pandemic. *European Journal of Cardiovascular Prevention and Rehabilitation*, Vol. 17.

Vit, P., Roubik, D. W., & Pedro, S. R. M. (2012). Pot-Honey: A legacy of stingless bees. In *Pot-Honey: A Legacy of Stingless Bees*.

Vowden, K., & Vowden, P. (2014). Understanding exudate management and the role of exudate in the healing process. *British Journal of Community Nursing*, *8*(Sup5).

Vyhlídalová, D., Kozáková, R., & Zeleníková, R. (2018). Management of non-healing wounds with honey dressings: A literature review. *Central European Journal of Nursing and Midwifery*, Vol. 9, pp. 880–888.

Yaacob, M., Rajab, N. F., Shahar, S., & Sharif, R. (2017). Stingless bee honey and its potential value: a systematic review. *Food Research*, *2*(2), 124–133.

Yimam, A., Hailu, A., Murugan, R., & Gebretensaye, T. (2021). Prevalence of diabetic foot ulcer and associated factors among diabetic patient in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. *International Journal of Africa Nursing Sciences*, *14*, 100285.

Zainol, M. I., Mohd Yusoff, K., & Mohd Yusof, M. Y. (2013). Antibacterial activity of selected Malaysian honey. *BMC Complementary and Alternative Medicine*, *13*(1), 129.

Zhang, P., Lu, J., Jing, Y., Tang, S., Zhu, D., & Bi, Y. (2017). Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis†. *Annals of Medicine*, Vol. 49.

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