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ANTIMICROBIAL PROPERTIES OF SOUTH AFRICAN HONEYS AGAINST PATHOGENS ASSOCIATED WITH WOUNDS

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of

Master of Science (Medicine) (Pharmaceutical Affairs)

Supervisor:

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Johannesburg, 2014

DECLARATION

I, Fharzana Khan, declare that this research report is my own work. It has been submitted in partial fulfilment for the degree of Master of Science (Medicine) (Pharmaceutical Affairs) in the Faculty of Health Sciences at the University of the Witwatersrand, Parktown, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this or any other University.

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This 12th day of March 2014

RESEARCH OUTPUTS

PUBLICATIONS

Antimicrobial properties and isotope investigations of South African Honey

Fharzana Khan, Sandy van Vuuren, Jackie Hill, Sven Kaehler and Mike Allsop

(Submitted to Journal of Applied Microbiology, December 2013)

ABSTRACT

The use of honey for therapeutic applications against a wide range of ailments has been demonstrated since the primordial age. Although modern-day medicine has advanced to substantial heights, there are still major concerns with drugs such as antimicrobials due to the ever growing problem of drug resistance. Presently, antimicrobial resistance is rife in South Africa (SA), where apart from common pathogens developing resistance, instances of extreme drug-resistant tuberculosis are dominating headlines. Complementary and alternative medication (CAM) such as honey has become a popular alternative, as patients often perceive that these 'natural' preparations are superior to conventional medicine with a lower incidence of adverse reactions subsequently resulting in the increased utilisation of CAM preparations.

The aim of this study was to validate the antimicrobial efficacy of SA honey against pathogens associated with wound infections. Prior to this evaluation, an extensive review into wound pathology, conventional antimicrobial wound dressings and honey's antimicrobial potential was conducted to provide an appropriate background to this study. Evaluation of the potential antimicrobial properties of the various SA honeys from varying geographical locations within the country against a variety of common wound pathogens was determined *in vitro*. Antimicrobial activity was gauged by determining minumum inhibitory concentrations (MICs) by the agar dilution method. Commercially available Manuka honey was utilised as a control and antimicrobial activity of these samples ranged from 15.28-41.67%. The mean MICs of the SA honey samples tested ranged from 10.42-50.00% with honey sample 16-(FYNBOS/WC) displaying the most desirable antimicrobial activity with a mean MIC of 10.42±8.27%.

Combination studies using selected honey samples having highest antimicrobial efficacy, with conventional antimicrobials such as ciprofloxacin, gentamicin and antifungal agents such as amphotericin B and nystatin, were performed. This was conducted to investigate whether the phenomena of synergism, additive or antagonistic effects were observed. Honey displayed noteworthy potential to be combined with antibiotics namely; ciprofloxacin and gentamicin and antifungals namely; nystatin to produce synergism. Synergism of 16-(FYNBOS/WC), 18-(MIXEDGUM/FS), 19-(CITYMIX/FS), 26-(FYNBOS/WC) and 41-(INDIGENOUS/WC) with gentamicin against *Stapyhlococcus aureus* (*S. aureus*) was most noticeable, displaying a sum of fractional inhibitory concentration (ΣFIC) of 0.27.

The physicochemical properties of selected SA honeys were further investigated with emphasis being placed on water content, sugar content and pH and how these particular properties affected its antimicrobial efficacy. Furthermore, levels of impurification of honey were also investigated. The pH of honey samples tested in this study ranged from 3.89 to 5.09, displaying acidic characteristics. The sugar content range was 77.00-82.50%, and the moisture content range was 15.80-21.60%. The percentage impurification ranged from 0.19-33.60% with 3 samples; [1–(CITYMIXA/EC), 47-(SALIGNAGUM/KZN) and 53-(LITCHI/MP)] demonstrating impurification that should further be investigated. No definite correlation was established between these physicochemical properties and the antimicrobial activity of honey.

The positive ramifications of this study formed a basis for expanding honey-based antimicrobial treatment against certain infectious diseases such as those affecting the skin. The study proved that harnessing the antimicrobial potential of SA honey could lead to positive outcomes especially in the SA setting from which these samples were derived.

ACKNOWLEDGEMENTS

In completion of this work, I would like to convey my sincerest gratitude and appreciation to various individuals that have assisted and guided me towards this goal.

My sincerest and everlasting gratitude and appreciation goes to God, without whom, nothing is possible.

To my parents, Mohamed Sayed Ebrahim and Logie Khan. Thank you for believing in me, trusting me and allowing me to pursue this venture. Thank you for every sacrifice you have made, your insurmountable support and your unconditional love. Mere words could never describe your efforts in providing me with only the best.

Thank you to my sister Fharnisa Khan. You have been such an important part of my life and have inspired me in so many ways. Your generosity and willingness to go out of your way for me, are second to none. In the same light I would like to thank my brother in law, Naveshen Naidoo for all you support and guidance over the years.

To my aunts Savy Ram and Moga Govender, your encouragement, constant words of support and love for me have helped me through some of my darkest days. Thank you for everything.

Thank you to my gran, Poobathy Govender. You instilled the value of education in me and nurtured me from my younger years up until today.

To the rest of my family, I cherish your invaluable support and love. You have inspired me in so many ways. Thank you.

My sincere gratitude goes towards my Supervisor, Professor Sandy van Vuuren. Your dedication, discipline, passion and willingness to succeed are unrivalled. The knowledge and motivation I have gained from you cannot be surpassed. Thank you.

Thank you to Kim Morgado and Mike Allsop for the provision of the honey samples utilised in this study.

Thank you to Jackie Hill and Sven Kaehler from Rhodes University for your tireless efforts in assisting me with the physicochemical analysis of my samples.

My sincerest appreciation goes to Olga Kunene, the laboratory technician, for your willingness to help without question. You bent over backwards in assisting me with my research and for that I am highly grateful.

My sincerest appreciation goes to Hamisha Soma for your contribution towards this research.

To Thiresen Govender, I extend my deepest gratitude to you for your invaluable input towards this research report and your proof reading thereof.

Thank you David Bayever for moulding me into the pharmacist I have become.

Thank you to all my friends, colleagues and every person that has had an influence on my life. It is impossible to single out each and every single one of you without involuntarily omitting one of you.

A special mention must be made to a group of people who have been my backbone of support and cornerstone to my success. Thank you, Alicia Munian, Ashley Naicker, Ceceila Tjale, Cristy Moodley, Deshnee Naidoo, Dipesh Daya, Elzaan Human, Laetitia Ngandu, Magashvaree Govender, Mokesh Mathevula, Nonkululeko Mashabane, Raeesa Moosa, Rutendo Mushuku, Saajidah Kara, Shaista Sing, Sugantha Bheem, Thiresen Govender, Unathi Mabona and Zainab Aziz. Thank you for catalysing the days I thought that would never end, for sharing in my joys and sorrows and for the remarkable experience over the past few years.

DEDICATION

This research report is dedicated to my parents Sayed and Loga Khan

The sacrifices you have made and the support you have afforded me will forever linger in my heart

This research report is also dedicated to my adorable niece Leyah Naidoo

A smile so bright, you could light up the darkest room

"It Always Seems Impossible Until It's Done"

Nelson R. Mandela

1918 – 2013

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LIST OF ABBREVIATIONS

AGD	Antimicrobial gauze dressing
AICS	Antibiotic-impregnated collagen sponges
AOAC	Association of official analytical chemists
ATCC	American type culture collection
CAM	Complementary and alternative medicines
CFU	Colony forming units
°C	Degrees celsius
ΣFIC	Sum of fractional inhibitory concentration
g	Gram
HIV	Human Immunodeficiency Virus
INT	Iodonitrotetrazolium violet
L	Litre
MRSA	Methicillin-resistant *Staphyloccus aureus*
mg	Milligram
MIC	Minimum inhibitory concentration
mL	Millilitre
N/A	Not applicable
NCCLS	National Committee for Clinical Laboratory Standards
NES	Not enough sample for analysis
PHMB	Polyhexamethylenebiguanide
SA	South Africa
SAMF	South African Medicines Formulary
TB	Tuberculosis
TSA	Tryptone soya agar
TSB	Tryptone soya broth
WHO	World Health Organisation

CHAPTER 1
INTRODUCTION

1.1 Background to this study

There has been an increase in the consumer use of complementary and alternate medicines (CAM) as this form of pharmacotherapy is perceived to be superior by patients. Furthermore, it is documented that a large number of South Africans (approximately 70%) use CAM to initiate therapy. Possible reasons for a more natural treatment regimen include reduction of undesirable side effects of conventional medications. Patients perceive that CAM is associated with minimal side effects when compared to conventional medication (Kumari et al., 2009). In addition, the cost implications may also be a strong motivator (Debas et al., 2006). The increased scientific need and consumer use of CAM has subsequently led to a resurgence in using these agents for medical ailments.

The continuous use of antibiotics in clinical practice has consequently resulted in the development of multiple antibiotic resistance among bacteria. This has resulted in scientific efforts to discover alternative therapeutic agents (Tan et al., 2009). Clinical studies and research have demonstrated the effectiveness of honey's therapeutic potential in treating wound infections. Honey has been utilised therapeutically since ancient times for a wide range of treatments related to infectious diseases. These include respiratory disorders, gastrointestinal disorders and infective conjunctivitis and more frequently for the treatment of wound infections (Molan, 1999 (a); Al-Waili, 2004; Basualdo et al., 2007; Tan et al., 2009). Famous ancient physicians such as Aristotle (384-322 BC) and Dioscorides (c.50 AD) commonly used honey as a dressing for wounds and ulcers (Molan, 1999 (b)). Honey has also been documented for its use in fatigue, sore throats, earache, toothache and vertigo (Molan, 1999 (b), Meda et al., 2004). Of particular interest is the fact that honey has demonstrated *in vitro* antimicrobial activity (Anyanwu, 2012; Moussa et al., 2012; Shenoy et al., 2012). Furthermore, various clinical studies have confirmed honey's capacity as an effective wound healing agent (Visavadia et al., 2006).

In particular, Manuka honey shows significant antimicrobial activity against various wound organisms as well as antibiotic resistant bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) (Visavadia et al., 2006; Mandal et al., 2010). Currently in Australia and New Zealand, Medihoney® and Manuka honey are marketed as therapeutic honeys suitable for use in ulcers, infected wounds and burns. Multifarious contemporary research has indicated that both these honeys, derived from the *Leptospermum* spp., have

significant antibacterial activity (Kwakman and Zaat, 2012; Kronda et al., 2013; Muller et al., 2013).

1.2 Wound pathology

The purpose of this study was to primarily investigate the antimicrobial impact of honey on pathogens associated with wounds. It was thus imperative to understand the fundamentals of wound infection and healing before embarking on the analysis of honey as an antiinfective wound healing agent.

The role of the skin as a significant barrier between the host and the environment cannot be overestimated as it protects the underlying tissue from becoming colonised and invaded by potential pathogens (Bowler et al., 2001). A wound is caused by the loss of skin integrity resulting in the exposure of subcutaneous tissue. Types of wounds vary and include; traumatic wounds, surgical wounds, burns and chronic wounds. There is an increased susceptibility to infection after the occurrence of a wound, and this is dependent on the access of pathogens and the immunocompetency status of the individual. A wound provides an environment which is conducive to microbial colonisation and subsequent infection as it is an ideal medium for a wide variety of micro-organisms. Wound contaminants are likely to originate from three sources. The environment can be an exogenous source as a result of micro-organisms in the air or from trauma. Endogenous sources usually involve mucous membranes (primarily the gastrointestinal, oropharyngeal and genitourinary membranes), and lastly contaminants can arise from normal skin microflora. Infection occurs when these contaminants, that are continuously multiplying, overwhelm the body's immune system resulting in inflammation and active disease. This, in turn, inhibits the healing of the wound. In severe infections, the contaminants may enter the bloodstream causing septicaemia which can result in organ failure and death (Bowler et al., 2001; Patel, 2007). Figure 1.1 is a typical illustration of a wound, which can be infected with a number of polymicrobial contaminants such as *Staphylococcus epidermidis, Corynebacterium* spp., *Proprionibacterium* spp., *Pityrosporum* spp., *Staphylococcus aureus, Beta-hemolytic Streptococcus, Escherichia coli, Proteus* spp*., Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter* spp., which may result in septicaemia. The inflammatory response is also demonstrated.

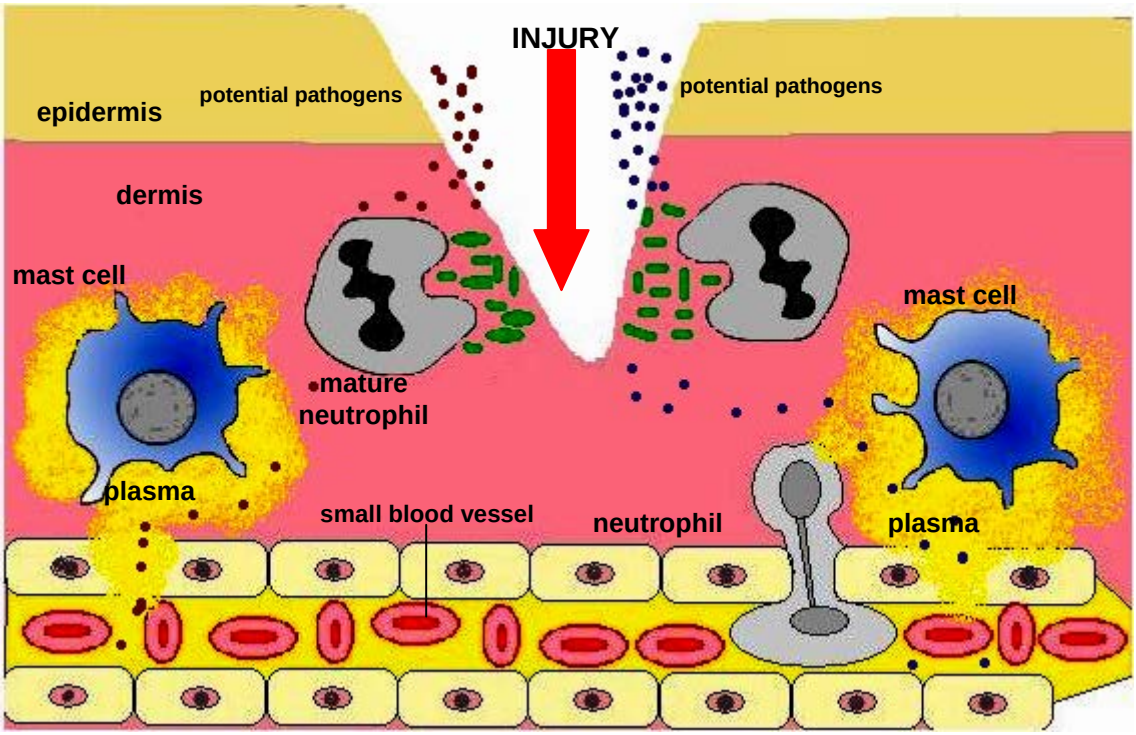


Figure 1.1: Illustration of a typical wound, the inflammatory reaction and the overwhelming contaminants resulting in septicaemia, [Adapted and modified from Dr D. Honardoust (The Canadian Association of Medical Spas and Aesthetic Surgeons). http://www.camacs.ca/Directors.php, accessed 20th December 2012].

Wounds have become a clinical concern and alternate agents could be beneficial. Honey has therapeutic properties as mentioned previously and research demonstrates its potential as a therapeutic agent especially in wound management. Silver sulfadiazine and mupirocin are antimicrobial agents conventionally utilised in the management of burns and wounds. A major shortcoming of topical antimicrobial agents is the fact that they increase the risk of developing antimicrobial resistant organisms (Ovington and Eisenbud, 2004).

Wound healing revolves around the body's replacement of injured tissue with healthy living tissue. The goals of wound therapy and management include; rapid wound closure and an aesthetically acceptable scar. Ideally, wound dressings should possess traits such as; absorbing exudates and potential toxins from the wound surface, allowing gaseous exchange, providing thermal insulation to the wound, maintaining a high humidity at the wound/dressing interface, being non-toxic, allowing for easy removal devoid of trauma, possessing considerable physicomechanical strength to resist damage and tear upon handling, being comfortable and sterilizable and protecting the wound from bacterial penetration (Turner, 1979; Stashak et al., 2004; Thu et al., 2012).

1.3 Conventional antimicrobial wound dressings

Various dressings exist for both acute and chronic wounds. Hydrogel-based dressings or hydrocolloid dressings have formed the platform for moist wound care. Wound healing is generally facilitated by dressings that maintain a moist environment. Hydrocolloid dressings function by forming a gel upon exposure to wound exudates. Gel formation removes excess fluid without leading to desiccation of the wound. A shortfall of hydrocolloid dressings is its fluid-handling capacity which is dependent on a number of factors including the physicochemical properties of the dressing (Thu et al., 2012). As bacteria are rife in wound fluid, it is pivotal that fluid-retaining dressings absorb and retain the bacteria present (Wysocki et al., 2002).

Depending on the type of wound, a drug-loaded wound dresssing such as an anti-infective or pain controlling dressing, may be used. These dressings are common in chronic wound therapy (Lawrence, 1994; Steffansen and Herping, 2008; Fouda et al., 2009). Antimicrobial wound dressings are utilised to minimise the number of micro-organisms. Due to the development of antibiotic-resistant organisms such as MRSA, vancomycin-resistant *Enterococcus faecalis* and *Pseudomonas aeruginosa*, there has been an increase in the use of antimicrobial dressings (Stashak et al., 2004). Critical colonisation of a wound leading to impaired wound healing necessitates the use of an antimicrobial dressing. Features of critical colonisation include: delayed healing, malodour, a new onset of pain and increasing slough even after debridement. Antimicrobial wound dressings generally contain silver, iodine and polyhexamethylenebiguanide (PHMB) and cover a wide range of micro-organisms including *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Abdelrahman and Newton, 2011).

The *in vitro* potential of silver chloride coated nylon dressings have been effectively demonstrated with the dressing displaying effective antimicrobial and antifungal action. Pathogens tested included *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Streptococcus equi* subspecies *zooepidemicus.* The dressing functions by the gradual release of silver over a period of time. Release rates of silver vary with different dressing types. It is proposed that the best time of use is from the inflammatory to repair phase of wound healing (Adams et al., 1999; Stashak et al., 2004). A major shortfall of the use of silver is its association with tissue toxicity and impaired healing.

Antimicrobial Gauze Dressing (AMD) (Kerlix®) is a gauze-based dressing containing the active ingredient PHMB. PHMB has a wide range of antimicrobial activity. The biocompatibility of the dressing is far more superior to chlorhexidine-based dressings,

reduces bacterial penetration into the wound and resists bacterial colonisation within the dressing itself. The dressing is best applied during the inflammation and debridement phases of the healing process. It is particularly advantageous in wounds with rife bacterial activity and an open synovial cavity. In addition, the dressing aids in drainage of the wound (Angelique and Rodeheaver, 2001; Stashak et al., 2004).

Iodine-based wound dressings synthesised from crosslinked polymerised dextran have been studied. Hydration of the dressing subsequently results in the release of iodine which has an antimicrobial effect and may interact with macrophages to indirectly facilitate wound healing. It is most efficacious in the early inflammatory and repair phases of wound healing. Similar studies have established iodine as a broad-spectrum antimicrobial with fungicidal activity (Moore et al., 1997; Hotoson-Moore, 2003).

Antibiotic-Impregnated Collagen Sponges (AICS) are of particular importance in orthopaedics and soft tissue surgery. They basically consist of a collagen platform interspersed with an antibiotic agent. A prime example of an AICS is CollatampG® consisting of gentamicin interspersed within a Type 1 bovine collagen that is denatured. The dressing operates by regulating homeostasis and causes aggregation and adhesion of platelets and a few bridge proteins. It prevents as well as treats infection by allowing the release of gentamicin from the collagen matrix. This is achieved by initial passive diffusion followed by collagen breakdown by macrophages. Drug concentrations are highest at the site of dressing occlusion (Letsch et al., 1991; Stemberger et al., 1997; Summerhays, 2000).

A poultice pad contains boric acid which is a mild antiseptic as well as Tragacanth which is the poultice agent. It is designed to fit the sole of the foot. It may be used in infected absecceses or dirt-infested wounds and its use may extend to other parts of the body (Stashak et al., 2004).

Activated charcoal wound dressings serve to facilitate wound healing by creating a moist environment, effectively absorb bacteria, prevent exhuberant granulation tissue formation and deodourise wounds. Antibacterial properties are solely attributed to its ability to absorb the bacteria thereby reducing their numbers (Frost et al., 1980).

Knill and co-workers (2004) synthesised alginated-based fibres treated with chitosan for use as an antibacterial wound dressing. The alginate served to absorb excess exudate and liquid and chitosan played a role in its antimicrobial, wound healing and haemostatic properties. In

addition, the chitosan was released in a slow, controlled manner providing extended antibacterial action (Knill et al., 2004).

Table 1.1 summarises and displays the current commercially available antimicrobial wound dressings in terms of their trade names, the active antimicrobial agent, the type of dressing supporting the active agent and the name of the manufacturers of the antimicrobial dressings (Zahedi et al., 2010).

Table 1.1: Commercially available antimicrobial dressings, [Adapted from Zahedi et al., 2010].

DRESSING NAME	ANTIMICROBIAL INGREDIENT	DRESSING FORMAT	MANUFACTURER
Acticoat absorbent	Ionic silver	Calcium alginate	Smith & Nephew, Inc, Largo, FL, USA
Actisorb Silver 220	Ionic silver and activated charcoal	Silver impregnated activated charcoal cloth	Johnson and Johnson Wound Management, Somerville, NJ, USA
Arglaes	Ionic silver	Transparent film or powder	Medicine Industries, Inc, Mundelein, IL, USA
Aquacel AG	Ionic silver	Hydrofiber	Convatec, Skillman, NJ, USA
Contreet H	Ionic silver	Hydrocolloid	Coloplast Corp, Marietta, GA, USA
Contreet F	Ionic Silver	Foam	Coloplast Corp, Marietta, GA, USA
Iodosorb	Molecular iodine	Gel or paste	HealthPoint Ltd, Ft. Worth, TX, USA
Silvasorb Antimicrobial Silver Dressing	Ionic silver	Hydrogel sheet or amorphous gel	Medline Industries, Mundelein, IL, USA
Kerlix AMD Gauze	PHMB	Gauze	Tyco Healthcare/Kendall, Mansfield, MA, USA

1.4 Honey as an antimicrobial agent

Investigations into the antimicrobial properties of honey from other geographical regions other than Southern Africa have been more substantially investigated. Studies have focused on a wide range of geographical regions including Dubai (Al-Waili et al., 2004), New Zealand (Brady et al., 2004) Australia (Lusby et al., 2005), Portugal (Henriques et al., 2005), Argentina (Basualdo et al., 2007), Wales (Cooper et al., 2008), Spain (Gallardo-Chacon et al., 2008), Iran (Khosravi et al.,2008), Ireland (Maeda et al., 2008) India (Mandal et al., 2010), Pakistan (Gulfraz et al., 2010), Chile (Sherlock et al., 2010), Cuba (Alvarez-Suarez et al., 2010) Malaysia (Khoo et al., 2010) Greece (Voidaroua et al, 2011), Algeria (Moussa et al., 2012), and Nigeria (Anyanwu, 2012) to name only a few. These studies provide an important global perspective as it is evident that the antimicrobial activity of honey varies according to their composition which in turn is dependent on geographical location, botanical origin, bee species, season, and its treatment since harvest including storage (Kaskoniene and Venskutonis, 2010). Furthermore, not all honeys are equally effective for wound healing as the antimicrobial activities of honey can demonstrate 100-fold variances (Sherlock et al., 2010).

A search using the keywords “honey as an antimicrobial” was performed utilising the renowned scientific database, ScienceDirect and was conducted to view results each year for the past 10 years until present day (November, 2013). The number of articles, each year, for the last 10 years is illustrated in Figure 1.2. The search summarises the vast world-wide data confirming honeys ability as a potential antimicrobial agent as well as the demonstration that honey research is escalating. In the last ten years, honey research has increased four-fold.

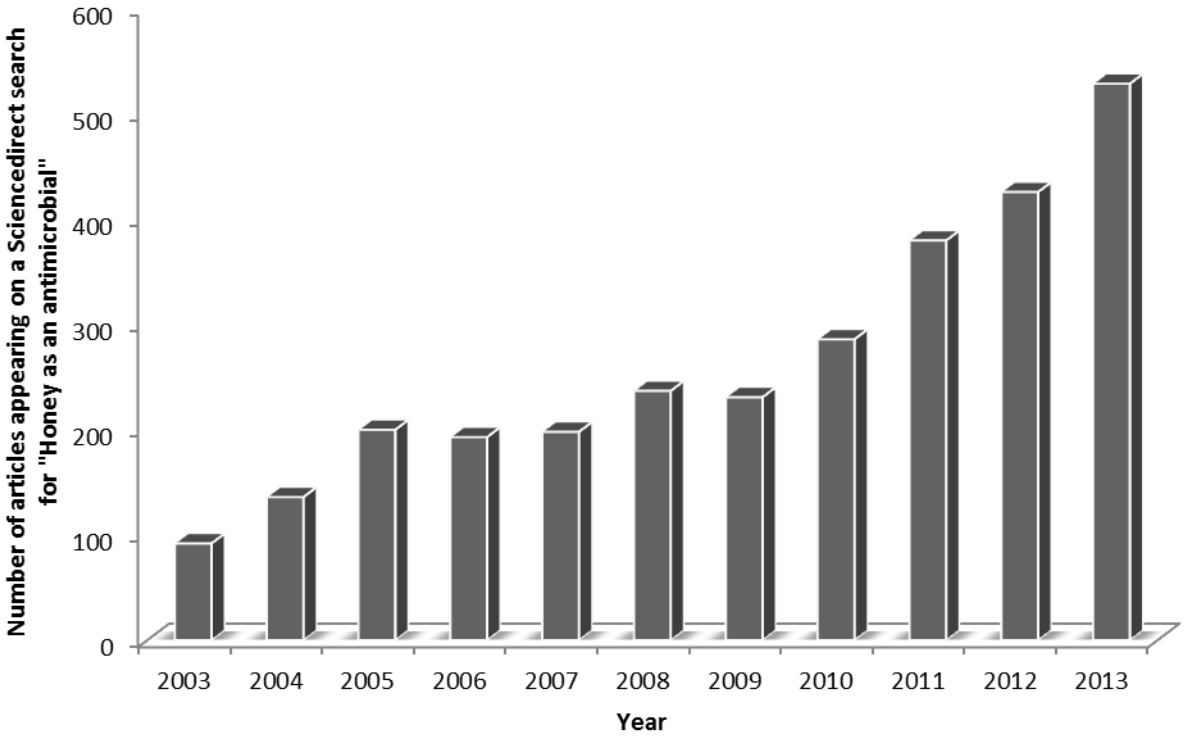


Figure 1.2 : Number of articles appearing on a ScienceDirect search using the key words “honey as an antimicrobial”, per year, over the last 10 years.

Various honey types have been studied as potential antimicrobial agents and a brief review of selected articles is outlined in Table 1.2. The table summarises only a proportion of the global perspective of honey research where the geographical region in which the honey(s) were harvested, the microbiological assay utilised, the tested pathogens and a brief summary of the results was obtained.

Table 1.2: Global antimicrobial research performed on honey.

GEOGRAPHICAL REGION OF HONEY	METHOD	PATHOGENS	RESULTS	REFERENCE
Cuba	Agar dilution method	*S. aureus, P. aeruginosa, B. subtilis, E. coli*	Most sensitive micro-organism was *S. aureus* followed by *P. aeruginosa*, *B. subtilis* and *E. coli* were moderately sensitive to the antimicrobial activity of honey. In general, the Gram-positive bacteria were more sensitive than the Gram-negative bacteria.	Alvarez-Suarez et al., 2010
Portugal	Agar streak dilution	*B. subtilis, S. aureus, S. lentus, P. aeruginosa, K. pneumoniae* and *E. coli*	*S. aureus* was the most sensitive strain and *B. subtilis, S. lentus, K. pneumoniae* and *E. coli* were moderately sensitive. *P. aeruginosa* displayed a lack of antimicrobial activity.	Estevinho et al., 2008
Greece	Agar well diffusion	*S. aureus*, *E. coli*, *S. typhimurium, S. pyogenes*, *B. cereus*, *B. subtilis*	Highest activity of 17.4 and 19.2% ($^{w}/_{v}$) was exhibited for coniferous and thyme honeys respectively. This was followed by citrus and polyfloral honeys with 20.8 and 23.8% respectively.	Voidaroua et al., 2011
Ireland	Broth dilution	MRSA	Honey was able to reduce the cultural count of all CA-MRSA from approximately 106 colony-forming units (CFUs) (mean=6.46 log10 CFU/g) to none detectable within 24 hours.	Maeda, et al., 2008
India, UK and New Zealand	Agar dilution	*E. coli* , *P. aeruginosa* and *S. enterica serovar Typhi*	The bactericidal activity of honey was demonstrated at 3% ($^{v}/_{v}$) for *S. enterica serovar Typhi* and *E. Coli* and at 3.5% ($^{v}/_{v}$) for *P. aeruginosa.*	Mandal, et al., 2010

GEOGRAPHICAL REGION OF HONEY	METHOD	PATHOGENS	RESULTS	REFERENCE
Algeria	Disc diffusion and well methods	*S. aureus, S. pyogenes*	The MIC% for *S. aureus* and *S. pyogenes* ranged from 12-95% and 25-73% respectively.	Moussa et al., 2012
Australia	Agar dilution method	*C. albicans, E. coli, Enterobacter aerogenes, K. pneumoniae, M. phlei, S. california, S. enteritidis, S. typhimurium, S. marcescens, S. sonnei, S. aureus* and *S. epidermidis.*	All honeys inhibited 12 of the 13 bacteria tested with only *S. marcescens* and *C. albicans* not inhibited. Although Medihoney® and Manuka honey had the overall best activity, the locally produced honeys had equivalent inhibitory activity for some of the bacteria.	Lusby et al., 2005
Argentina	Agar well diffusion	*S. aureus*, *S. epidermidis*, *M. luteus*, *S. uberis*, *E. faecalis*, *P. aeruginosa*, *E. coli*, and *K. pneumonia.*	*S. aureus* and *S. epidermidis* was inhibited by the majority of the undiluted honeys. Some honey samples provided by apiarists also inhibited the growth of *S. aureus* even at 50% dilution.	Basualdo et al., 2007
New Zealand	Agar well diffusion	58 strains of *S. aureus* were tested	MICs ranged from 2-3% ($^{v}/_{v}$) for Manuka honey and 3-4% ($^{v}/_{v}$) for pasture honey.	Cooper et al., 1999
Costa Rica	Agar well diffusion	*P. aeruginosa, S. aureus, S. cerevisiae, C. albicans and B. cereus*	The activity of honey produced by *Apis mellifera* and *T. angustula* was similar. In addition, the susceptibility of yeasts to honey of either species was greater than that of bacteria.	Demera and Angert, 2004

GEOGRAPHICAL REGION OF HONEY	METHOD	PATHOGENS	RESULTS	REFERENCE
Malaysia	Broth dilution	*S. pyogenes, coagulase-negative Staphylococci,* MRSA*, S. agalactiae and S. aureus, S. maltophilia, A. baumannii, S. enterica serovar typhi, P. aeruginosa, P. mirabilis, S. flexneri, E. coli and E. cloacae*	Tualang honey displayed MICs ranging from 8.75 - 25% when compared to Manuka honey with MICs ranging from 8.75-20%.	Tan et al., 2009
New Zealand	Agar incorporation (dilution) technique	Coagulase-negative *Staphylococci*	Inhibitory action at dilutions of 3.6 ± 0.7% ($^{v}/_{v}$) for the pasture honey and 3.4 ± 0.5% ($^{v}/_{v}$) for the Manuka honey	French et al., 2005
UAE	Broth dilution	*E. coli, E. cloacae, P. aeruginosa, S. dysenteriae, Klebsiella spp., H. influenzae, Proteus spp., S. aureus, S. hemolyticus* group B, and *C. albicans*	Growth of all the isolates was completely inhibited at honey concentrations ranging from 30–100% with the most sensitive microbes being *E. coli, P. aeruginosa,* and *H. influenzae*.	Al-Waili, 2004
Nigeria	Agar well diffusion	*A. niger, A. flavus, P. chrysogenum, M. gypseum, C. albicans* and *Saccharomyces spp.*	The most sensitive of all the fungal isolates studied was *M. gypseum,* while *C. albicans* was the least sensitive. The MIC and Minimum Fungicidal Concentration (MFC) values for the honeys ranged between 12.5-50% ($^{v}/_{v}$).	Anyanwu, 2012
Australia	Phenol equivalence assay (disc diffusion and well methods)	*S. aureus*	Exemplary activity was seen in samples derived from marri (*Corymbia calophylla*), jarrah (*Eucalyptus marginata*) and jellybush (*Leptospermum polygalifolium*).	Irish et al., 2011
Iraq	Disc diffusion method	*S. aureus, E. coli* and *P. aeruginosa*	Inhibitory effects *in vitro* at concentrations of 50, 75 and 100%.	Al-Naama, 2009

GEOGRAPHICAL REGION OF HONEY	METHOD	PATHOGENS	RESULTS	REFERENCE
New Zealand, Chile	Agar well diffusion	MRSA, *E. coli* and *P. aeruginosa*	Ulmo 90 honey displayed a greater antibacterial activity (MIC 3.1%-6.3% $^{v}/_{v}$) against all MRSA isolates tested and similar activity against *E. coli* and *P. aeruginosa* than Manuka honey (12.5% $^{v}/_{v}$).	Sherlock et al., 2010
Nigeria	Agar well diffusion	*S. aureus, P. aeruginosa, K. pneumoniae, B. subtilis* and *E. coli*	Inhibition of growth of the test organisms being achieved between 25-100% ($^{w}/_{v}$).	Osho and Bello, 2010
Czech Republic	Agar dilution Method	*L. monocytogenes, S. aureus, E. coli* and *S. typhimurium*	Honey produced in the Czech Republic is antimicrobially effective with the highest and most significant effect in honeydew honeys.	Vorlova et al., 2005
Egypt	Agar well diffusion	*P. aeruginosa, E. coli, K. pneumoniae, K. oxytoca, B. cereus, B. subtlis, B. pumilus, B. brochiseptica* and *M. luteus*	Undiluted honey samples displayed antimicrobial activity at 75%, 50%, 30% and 10% across all pathogens tested. Variations in antimicrobial activity were attributed to the phenolics; ferulic acid and pinobanksin isolated.	Hamouda and Marzouk, 2011
Algeria	Disc and well diffusion methods, spectrophotometric assay	*E. coli* and *P. aeruginosa*	Zone of inhibition diameter for tested honey for *P. aeruginosa* and *E. coli* was 0-30 mm and 0-38 mm, respectively. The MIC ranged from 90-91% and 56-96% for the respective organisms.	Moussa et al., 2012

GEOGRAPHICAL REGION OF HONEY	METHOD	PATHOGENS	RESULTS	REFERENCE
Iran	Microdilution broth method	*C. albicans, C. parapsilosis, C. tropicalis, C. kefyr, C. glabrata, C. dubliniensis*	*C. albicans* growth was stunted by increasing honey concentrations. The highest inhibitory effect of different honeys was reported in *C. tropicalis, C. glabrata and C. dubliniensis*. All honeys were able to produce complete inhibition of candidal growth with MFC ranging from 29-56 %.	Khosravi et al., 2008
Wales	Agar well diffusion	*S. aureus*	The total and non-peroxide activity of Manuka honey, was equivalent to 18.5% ($^{w}/_{v}$) phenol. 71 of the Welsh samples possessed a 6.9% $^{w}/_{v}$ highest phenol equivalent.	Cooper et al., 2010
New Zealand	Agar well diffusion	*S. aureus, C. albicans, E. coli, T. mentagrophytes*	Antibacterial activity against *S. aureus* was demonstrated by half of the samples tested with activity ranging from 5.0-27.9% phenol equivalent. Antibacterial activity against *E. coli* was evident in approximately 30% of the samples tested. Despite this, honey concentrations required for inhibition were in excess of 19%, with a single exception. Similarly, antifungal activity was observed in 35% of samples although the levels of activity measured were low.	Brady et al., 2004

GEOGRAPHICAL REGION OF HONEY	METHOD	PATHOGENS	RESULTS	REFERENCE
Pakistan	Agar well diffusion	*S. aureus, E. coli, C. albicans* and *A. niger*	The growth of bacteria, yeast and fungi were significantly inhibited by Acacia and Citrus honey. Undiluted or 100% honey proved to be the most suitable for the inhibition of growth of pathogenic organisms.	Gulfraz et al., 2010
India	Agar dilution method	*P. aeruginosa*	All the strains of *P. aeruginosa* were found to be sensitive to honey at an MIC of 20%. This was compared to Dettol® with an MIC of 10%. Depending on the dilutions of the honey tested, all the isolates of *P. aeruginosa* tested were killed in 12-24 h.	Shenoy et al., 2012
South Africa	Agar well diffusion	*H. pylori*	Activity against *H. pylori* was achieved by all the honeys with most activity at a concentration of 75% $^{v}/_{v}$. A zone of inhibition diameter of 18.0 ± 7.4 mm was achieved by clarithromycin, the positive control. This was not significantly different from honeys at 75% $^{v}/_{v}$ and solvent extracts.	Manyi-Loh et al., 2010

GEOGRAPHICAL REGION OF HONEY	METHOD	PATHOGENS	RESULTS	REFERENCE
South Africa	Broth dilution	*C. albicans, S. anginosus, S. oralis*	Carbohydrate concentration proved pivotal in the antimicrobial activity of the honeys above 25%. The antibacterial activity of the honeys was far superior to the antifungal activity of the honeys with the bacteria being more susceptible and the yeast, *C. albicans* more resistant. *S. anginosus* and *S. oralis* proved to be superiorly sensitive to the honeys than the other test bacteria.	Basson and Grobler, 2008
South Africa	Broth dilution method	*C. albicans*	A reduction in the growth of *C. albicans* was observed with increasing honey concentrations. Whilst partial inhibition was demonstrated with the controls, bluegum and fynbos, wasbessie honey at a concentration of 25% demonstrated 29.4% inhibition on the growth of *C. albicans.*	Theunissen et al., 2001

Given the global attention to this field of research, the lack of research conducted on SA honeys is of significant concern. SA possesses a significant floral biodiversity with many unique, indigenous plants. Furthermore, there are over 30 000 plant species of which the Cape boasts one of the most diverse temperate flora on earth which could potentially result in many types of unique honey with varying floral origins (Goldblatt and Manning, 2002). The honey industry in SA has a growing potential to produce honey of suitable quality and quantity, however, SA utilises approximately 2026 tons of honey annually of which only 1533 tons are produced in SA and the balance of 493 tons is imported (Department of Water Affairs and Forestry, 2005). China is one of the largest global producers of honey and produced 402 000 tons in 2009 (Guo-xue et al., 2011). Standardisation of honey may help stimulate and further nurture the honey industry in SA.

The antimicrobial efficacies of SA plants have been studied extensively with many promising antimicrobial properties (Van Vuuren, 2008). Furthermore, there is in excess of a 100 nectar and pollen producing plants in South Africa, all of which contribute significantly to honey production. At least 38 of these species are indigenous (Anderson et al., 1983). Bearing this in mind, there is the potential that some honeys may have superior or similar antimicrobial properties to the well marketed Manuka honey which is a *Leptospermum*-derived honey. Other *Leptospermum*-derived honeys include; Medihoney®, a therapeutic honey suitable for use in ulcers, infected wounds and burns currently marketed in Australia.

Research has indicated that these honeys, have significant antimicrobial activity against various wound organisms as well as antibiotic resistant bacteria (Brady et al., 2004). Despite this, relevant searches on globally accepted scientific databases Scopus, ScienceDirect and PubMed (search date - November 2013), reveal limited studies involving the antimicrobial investigations of SA honeys (Theunissen et al., 2001; Basson and Grobler, 2008; Manyi-loh et al., 2010; Manyi-loh et al., 2012) have been undertaken.

Manyi-loh and co-workers (2010) investigated the activity of commercially purchased honeys, Goldcrest, Pure Honey (floral sources of Citrus limon and Citrus sinesis) and Citrus Blossom (floral source of berry orchards) against *Helicobacter pylori*. All the honeys demonstrated activity against *H. pylori* at honey concentrations of 10.00% ($^{v}/_{v}$) and greater. Manyi-loh and co-workers (2012) further investigated the fraction responsible for antibacterial activity in Goldcrest n-hexane extract. The Goldcrest mobile phase 3 fraction (5mg/ml) displayed the best antibacterial activity. Basson and Grobler (2008) investigated the activity of a variety of honeys from *Eucalyptus cladocalyx* (bluegum), *Leucospermum cordifolium* (pincushion), a mixture of heather shrubs mainly *Erica* species (fynbos) and

Leptospermum scoparium (Manuka honey) against a number of *Streptococcus* strains as well as *Candida. albicans, Escherichia coli* and *Staphylococcus aureus.* These honeys demonstrated antimicrobial activity at concentrations of 50.00% ($^{v}/_{v}$). Theunissen and co-workers (2001) examined the antifungal activity of honey commonly found in the Western Cape region of SA against *C. albicans.* The floral types of honey investigated were *Eucalyptus cladocalyx* (bluegum), *Myrica cordifolia* (wasbessie) and fynbos (a mixture of many heather shrubs mainly derived from the botanical origin of *Erica* species). The study investigated concentrations up to 25.00% ($^{w}/_{w}$). It was demonstrated that an increased honey concentration resulted in a greater inhibition of *C. albicans.* It was also reported that *Myrica cordifolia* honey (25.00% $^{w}/_{w}$) produced the greatest inhibition against *C. albicans* (Theunissen et al., 2001).

While these studies demonstrate the antimicrobial potential of SA honeys, they unfortunately focused on a limited number of honey samples, with little attention given to possible impurification. Furthermore, none of the studies took cognisance of the fact that honey has historically been associated with wounds and as such, pathogens associated with wound healing could have been given priority. With this in mind, the study presented here focused on 83 SA honey samples and pathogens associated with wound healing.

1.5 Physicochemical properties of honey

The physicochemical properties of honey contribute to its antimicrobial and wound healing properties. Contributing properties are osmolarity, pH, water content and hydrogen peroxide production. Hyperosmolar activity has both bactericidal and bacteriostatic activity and functions as the high solutes in the honey draws the water required for growth by the pathogens and cause shrinkage and destruction of the bacterial cell wall (Bangroo et al., 2005). The enzyme glucose oxidase is incorporated into honey by the bee during nectar processing and is responsible for the conversion of glucose to gluconic acid and hydrogen peroxide upon the addition of water. Research has indicated that an acidic pH (3.2-4.5) contributes to antimicrobial activities (Mandal et al., 2010). The pH of honey aids in creating optimal conditions for fibroblast activity, allowing the migration, proliferation, and organisation of collagen and increases the quantity of oxygen off loaded from haemoglobin into the capillaries. Furthermore, the high viscosity of honey helps to provide a protective barrier to prevent infection (Lusby et al., 2002; Simon et al., 2009). The benefits of a moist environment are well recognised in wound healing, where wound repair is accelerated. The moist environment offered by honey protects the wound, reduces infection rates, debrides

necrotic tissue, promotes granulation tissue formation and is an effective analgesic (Lusby et al., 2005).

Figure 1.3 summarises the pH conditions for optimum wound healing. In essence, a lower pH results in wound healing whereas an increased pH results in wound breakdown. The schematic emphasises the fact that a pH of near 3.5 (the pH of Medihoney®) aids in wound healing by significantly reducing the surface wound pH, thus increasing oxygen diffusion and decreasing protease activity. Medihoney® facilitates the overall wound healing process by promoting a moist wound environment, reducing inflammation, debriding the wound and stimulating the immune system.

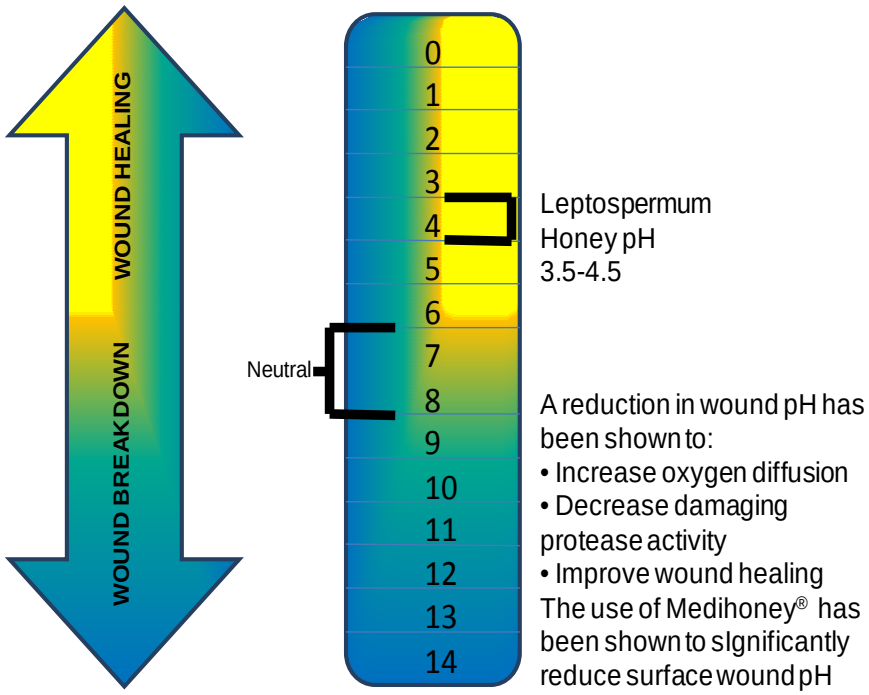


Figure 1.3: The pH conditions for optimum wound healing, [Adapted from www.mountainside-medical.com/products/Medihoney-Honeycolloid-Dressing-with-Adhesive-Border-10%7B47%7Dbox.html, accessed 20th January 2013].

1.6 Wound dressings containing honey

An overview of wound dressings (Section 1.3) demonstrates the importance of relevant treatment protocols in wound management. The use of honey in wound dressings has also been investigated. Honey-based hydrogel dressings have also been formulated for enhanced wound healing (Yousof et al., 2007; Wang et al., 2012).

Wounds dressed with honey often show early initiation of anti-inflammatory activity and stimulation of immune responses (Brady et al., 2004). The proliferation of B-lymphocytes and T-lymphocytes, tumour necrosis factor, and interleukin factors have been demonstrated by honey (Molan, 2001). The anti-inflammatory action of honey reduces oedema and the amount of exudates produced. Re-epithelisation and growth of granulation tissue occur quicker resulting in quicker rates of wound healing and decreased scarring (Molan, 2006; Simon et al., 2009).

Honey aids in the deodorisation of infected wounds. Amino acids from the serum and dead cells are normally metabolised by bacteria resulting in the formation of ammonia, amines and sulphur compounds. These compounds are generally responsible for the malodour associated with wounds. Honey provides a substitute to the amino acids resulting in the formation of lactic acid (Lusby et al., 2002). Honey is also a non-adherent dressing as the layer of diluted fluid and honey prevents the dressing from adhering to the wound, thus the dressing can be changed without disrupting the partially healed wound or causing tenderness to the patient.

The desirable properties of honey make it an ideal marketable wound healing product, however, honey as with most natural products, may have a larger variance in therapeutic components depending on its origin. Not all honeys are equally effective for wound healing as the antimicrobial activities of honey can demonstrate 100-fold variances (Sherlock et al., 2010). Honey comprises a wide range of constituents including a range of carbohydrates, water, amino acids, enzymes, organic acids, phenolic acids, flavonoids, pollen ash and wax (Henriques et al., 2005). Variations in the ratios of these constituents exist in different types of honey depending on botanical origin, age, season, climate and its treatment since harvest.

Molan (2006) successfully corroborated various studies establishing honey as an effective antimicrobial agent especially useful in wound management (Molan, 2006). The antibacterial potential of honey has been demonstrated against a variety of Gram-positive and Gram-negative pathogens with activity extending to aerobic and anerobic organisms (Abdelmalek et al., 2012). The contemporary use of honey in wound dressings is an innovation that is fast-developing.

European Patent EP1450871B1 (Bray et al., 2009) describes a formulation that is a wound dressing. The dressing consists of a wound contact layer containing honey (antimicrobial) and sodium alginate (moisture absorbing), a fabric backing layer that is water-permeable

and an intermediate layer, that is also water-permeable and contains honey and sodium alginate. A diagrammatic representation of the patented honey-based dressing is illustrated in Figure 1.4 and emphasises the novelty and importance of utilising honey as an antimicrobial agent for wound management (Bray et al., 2009).

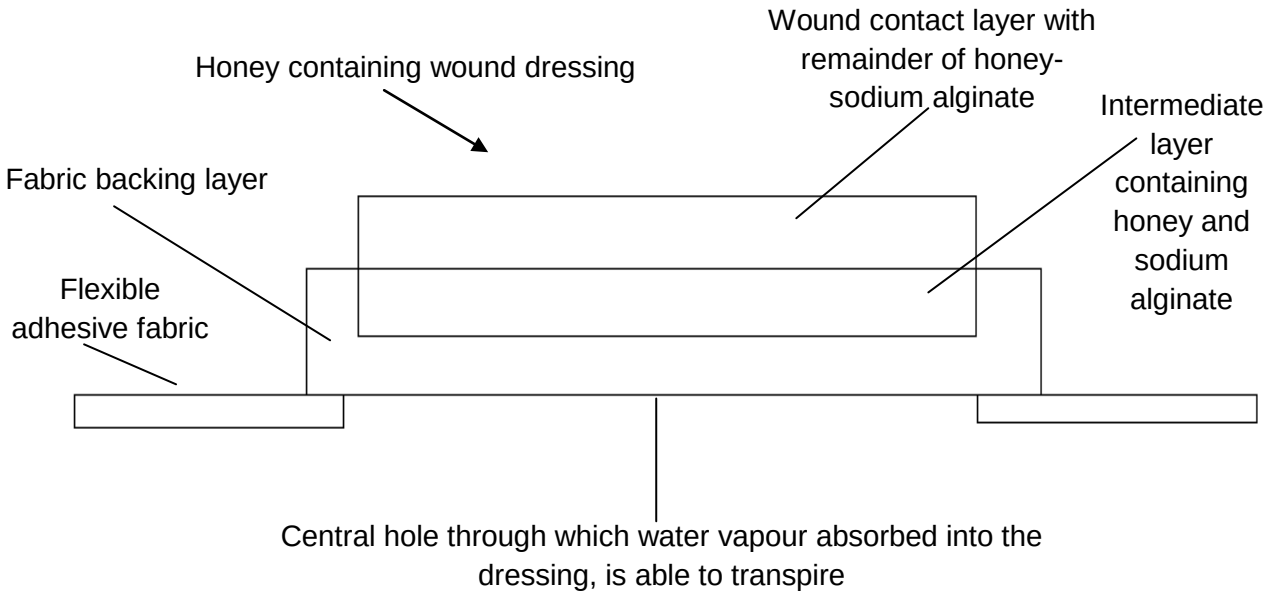


Figure 1.4: Diagrammtic representation of a honey-containing wound dressing, [Adapted from Bray et al., 2009].

1.7 Honey derived from *Leptospermum scoparium*

After extensive research on many unifloral New Zealand honeys, the greatest activity was noted with Manuka honey derived from the native Manuka tree, *Leptospermum scoparium.* The non-peroxide antimicrobial activity of honey is found to be due to components derived from the floral source. It was also established that not all Manuka honeys shared the same antimicrobial activity and activity varied with differing localities. Manuka honey has been demonstrated to be effective against a wide number of pathogens including *H. pylori, S. enterica serovar Typhi, P. aeruginosa, E. coli* and MRSA (Somal et al., 1994; Maeda et al., 2008; Mandal et al., 2010).

Medical honey containing antimicrobial agents are active against a wide spectrum of pathogens when undiluted. Upon dilution, however, the activity of these honeys is not the same (Cooper and Jenkins, 2009). Methylglyoxal is a distinctive heat stable component of Manuka honey which allows the honey to retain its antimicrobial activity even when diluted with wound exudates (Mavric et al., 2008). The mechanism of antimicrobial action of Manuka

honey centres around the honey's ability to inhibit bacterial cell division and disrupt the bacterial cell surface, thus leading to cell lysis and death (Henriques et al., 2010; Jenkins et al., 2011).

Honeys produced from *Leptospermum* spp. are commercially available as the Medihoney® antibacterial range of honey-based wound dressings. Medihoney® dressings are indicated for wounds colonised by antibiotic-resistant pathogens, locally infected wounds, chronic wounds, necrotic wounds, sloughy wounds, burns, donor and recipent graft sites, superficial wounds, surgical wounds and malodorous wounds. Although Medihoney® is absorbed by the body, the amount absorbed does not raise the blood sugar and hence it is safe for use by diabetic patients. In addition, Medihoney® is non-toxic and may be used in paediatric patients. Another significant advantage of Medihoney® is the fact that there are no known reports of bacterial resistance (Grothier and Cooper, 2011).

Figure 1.5 describes the mechanism of action of Medihoney®. The wound is kept moist by the osmotic pull of Medihoney® drawing the lymph from the deeper tissues through the wound. Removal of devitalised tissue from the wound bed is facilitated by the outflow of lymph fluid.

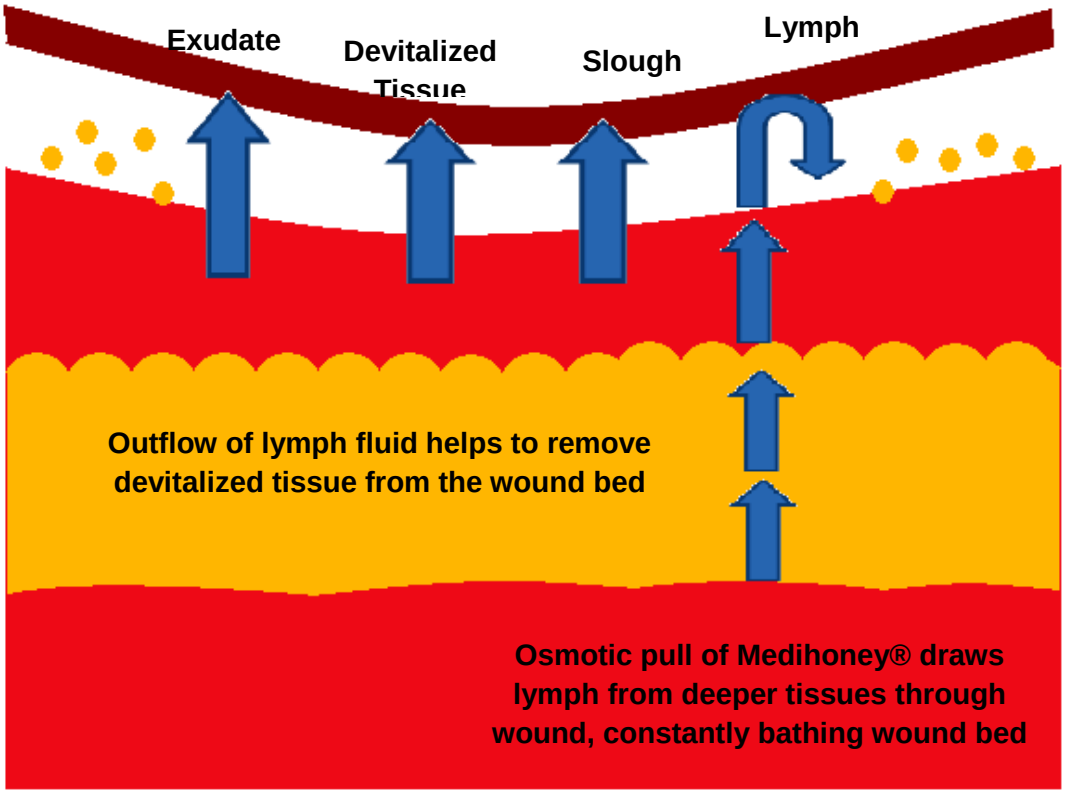


Figure 1.5: Mechanism of action of Medihoney® [Adapted from www.mountainside-medical.com/products/Medihoney-Honeycolloid-Dressing-with-Adhesive-Border-10%7B47%7Dbox.html, accessed 20th January 2013].

1.8 Honey combinations and synergy

Infectious diseases impact on human health and mortality. Effective treatment of these diseases is becoming more difficult as a result of emerging antimicrobial resistance and the lack of development of new antimicrobial drugs poses a threat to healthcare. To combat these problems combination therapy is often practiced. Combination therapy is the gold standard in the treatment of many infectious diseases such as Human Immunodeficiency Virus (HIV) and Tuberculosis (TB). Honey is one complementary medicine that can be potentially synergistic with conventional antimicrobial therapies and aid in combating bacterial resistance. A handful of combination studies bears testament to this (Table 1.3). This study investigated the potential of combining SA honey with other conventional wound agents to produce a possible synergistic effect.

Table 1.3: Combination studies performed with honey and conventional antimicrobial agents.

COMBINATION	METHOD	PATHOGEN	RESULTS	REFERENCE
Manuka honey and oxacillin	Disc diffusion, E test strips, serial broth dilution, chequer boards	MRSA	Synergism was observed between oxacillin and Manuka honey against MRSA	Jenkins and Cooper, 2012
Medihoney® and rifampicin	Checkerboard microdilution assays, time-kill curve experiments, agar diffusion	MRSA	Synergism was observed between Medihoney® and rifampicin against MRSA and *S. aureus*	Muller et al., 2013
Omani honey and gentamicin	Agar well diffusion	*S. aureus*	The rate of eliminating micro-organisms was greater when honey was combined with gentamicin, enhancing gentamicin activity by 22%.	Al-Jabri et al., 2005
Honey of Indian origin and gentamicin, amikacin and ceftazidime	Broth dilution	*Pseudomonas* spp*., Klebsiella* spp*.*	Combinations of honey and gentamicin, amikacin and ceftazidime individually demonstrated synergy against *Pseudomonas* spp*.*	Karayil et al., 1998

COMBINATION	METHOD	PATHOGEN	RESULTS	REFERENCE
Manuka honey and amoxicillin, penicillin G, cephalexin, ceftizoxime, colistin, erythromycin, gentamicin, imipenem, kanamycin, mupirocin, piperacillin/tazobactam, ciprofloxacin, rifampicin, tetracycline and vancomycin	Disc diffusion, E test strips, serial broth dilution, chequer boards, growth curves	*P. aeruginosa* and MRSA	Synergistic activity between Manuka honey and each of tetracycline, imipenem and mupirocin were observed against MRSA and additivity between each of Rifampicin, tetracycline and colistin and Manuka honey against *P. aeruginosa*	Jenkins and Cooper, 2012
Honey and imipenem, ciprofloxacin, amoxicillin/clavulanic acid, sublactam/ampicillin, ceftriaxone, amikacin, aztreonam, vancomycin, methicillin	Disc diffusion	*P. aeruginosa, coagulase-positive Staphylococci, Enterobacter* spp*., Klebsiella* spp*.*	The combination of honey and antibiotic demonstrated a significant increase in the mean inhibition zones that was greater for honey alone. Exceptions were noted for amikacin and aztreonam.	Abd-El et al., 2007

1.9 Study aims and objectives

The aim of this study was to investigate the antimicrobial activity of SA honeys against potential wound pathogens. In addition, combination studies with conventional antibiotics as well as the physicochemical properties of these honeys were also investigated.

In order to achieve this aim, the following objectives were outlined:

1. Analysis of the antimicrobial minimum inhibitory concentration (MIC) of 83 honey samples utilising the agar dilution method.
2. Analysis of the physicochemical properties of the honeys including pH, water content and sugar content. Impurification/adulteration was also investigated. Physicochemical properties were also correlated with antimicrobial activity.
3. Analysis of the combined effect of honey with conventional antimicrobial medicines utilising the fractional inhibitory concentration (FIC) index was carried out to determine if the honey and these agents are synergistic, indifferent or antagonistic when investigated in combination.

1.10 Potential benefits of this study

1. Identification of South African honeys with potential antimicrobial activity.
2. Emphasis on the synergistic or additional benefits of combining SA honey with conventional antimicrobial agents.
3. Possible decrease in the incidence of antimicrobial resistance by combining conventional antimicrobial agents with honey displaying antimicrobial activity.
4. The study may be utilised as a strong motivator for export or promotion of the SA honey as a therapeutic ‘medi-honey’.
5. Creating a platform for expanding ‘honey-based’ wound care to other complementarty agents thereby alleviating the constant use of conventional antimicrobial.

1.11 Overview of this research report

Chapter one provides an introduction and background to this study and outlines the challenges faced with wound management and conventional antimicrobial therapy. Conventional topical wound therapy in terms of antimicrobial wound dressings and the use of honey as an antimicrobial agent for primary wound care is also reviewed. This chapter highlights the rationale, aim, objectives and potential benefits of this study.

Chapter two investigates the antimicrobial properties of SA honeys with respect to pathogens associated with wound infections utilising the minimum inhibitory agar dilution assay.

Chapter three investigates the antimicrobial effect of honey in combination with conventional antibiotics and antifungal agents to assess if antimicrobial effects are additive, synergistic, indifferent or antagonistic.

Chapter four assesses the physicochemical properties of the honey samples as pH, water content and sugar content are known to impact antimicrobial activity.

Chapter five provides the conclusion of this research report and discusses limitations, recommendations and future investigations.

CHAPTER 2

INVESTIGATION INTO THE ANTIMICROBIAL ACTIVITY OF VARIOUS SOUTH AFRICAN HONEYS

2.1 Introduction

World-wide research has been performed on honey however there is currently very little data existing with regards to SA honey. In order to scientifically validate the traditional therapeutic use of honey as a wound dressing, the antimicrobial activity of various SA honeys was investigated. Extensive antimicrobial testing against a variety of common wound pathogens was conducted to determine the minimum concentration of honey that can successfully inhibit the growth of these pathogens tested in this study.

Evaluation of the potential antimicrobial properties of the various SA honeys revolved around rigorous *in vitro* testing.

2.2 Materials and methods

2.2.1 Acquisition of honey samples

In collaboration with Kim Morgado (beekeeper in Johannesburg) and Mike Allsopp from the Honey Research Station, Agricultural Research Council in Stellenbosch, a variety of un-heated, non-irradiated, un-clarified as well as un-processed honey samples from various botanical sources throughout SA (Eastern Cape, Western Cape, Kwa-Zulu Natal, Mpumalanga, North West, Limpopo, Free State) were collected. All honeys varied in colour, viscosity, taste and smell (Figure 2.1).



Figure 2.1: Some of the varieties of honeys acquired from various botanical sources across SA.

Samples were collected from December 2005 to September 2011 in order to accommodate different floral types and seasonal variation. Samples were sourced throughout South Africa as geographical location may influence the antimicrobial efficacy (Irish et al, 2011). A map indicating the geographical location of the honey samples is illustrated in Figure 2.2 which further illustrated that the majority of samples collected were from the Western Cape region. This is due in part to the vast floral biodiversity exhibited in the Cape region (Goldblatt and Manning, 2002) and the supplier residing in this region.

2.2.2 Processing, referencing and coding of honey samples

Honey samples were stored away from light and at room temperature. Honey types were identified and the perceived source (where possible) noted by the apiarists. The samples were numbered, and coded in order to reference and trace the respective samples (Table 2.1). In addition, the samples were recorded according to their scientific and common names, date of collection, and geographical location as per Table 2.1. The samples were numbered and coded in order to reference and trace the respective samples. Honey samples were labelled whereby a number according to order of receipt was issued followed by the perceived source (where possible) noted by the apiarists and thereafter a geographical code given based on the province where the samples were attained. The focus

of the current study was the antimicrobial analysis of 55 samples collected in the year of analysis (2011 (Table 2.2). Concentrations of honey were expressed as the percentage of honey volume per total volume ($^{v}/_{v}$). A subset of older honey samples (collected from 2005) from the same geographical region with perceived equivalent botanical source was then selected to analyse possible antimicrobial efficacy variations with ageing of honey as a key independent variable assessed. Seven samples from 2010 were analysed, three from 2009, sixteen from 2008, one from 2007 and one sample with details unknown was also evaluated as these details could not be provided by the supplier of the honey.

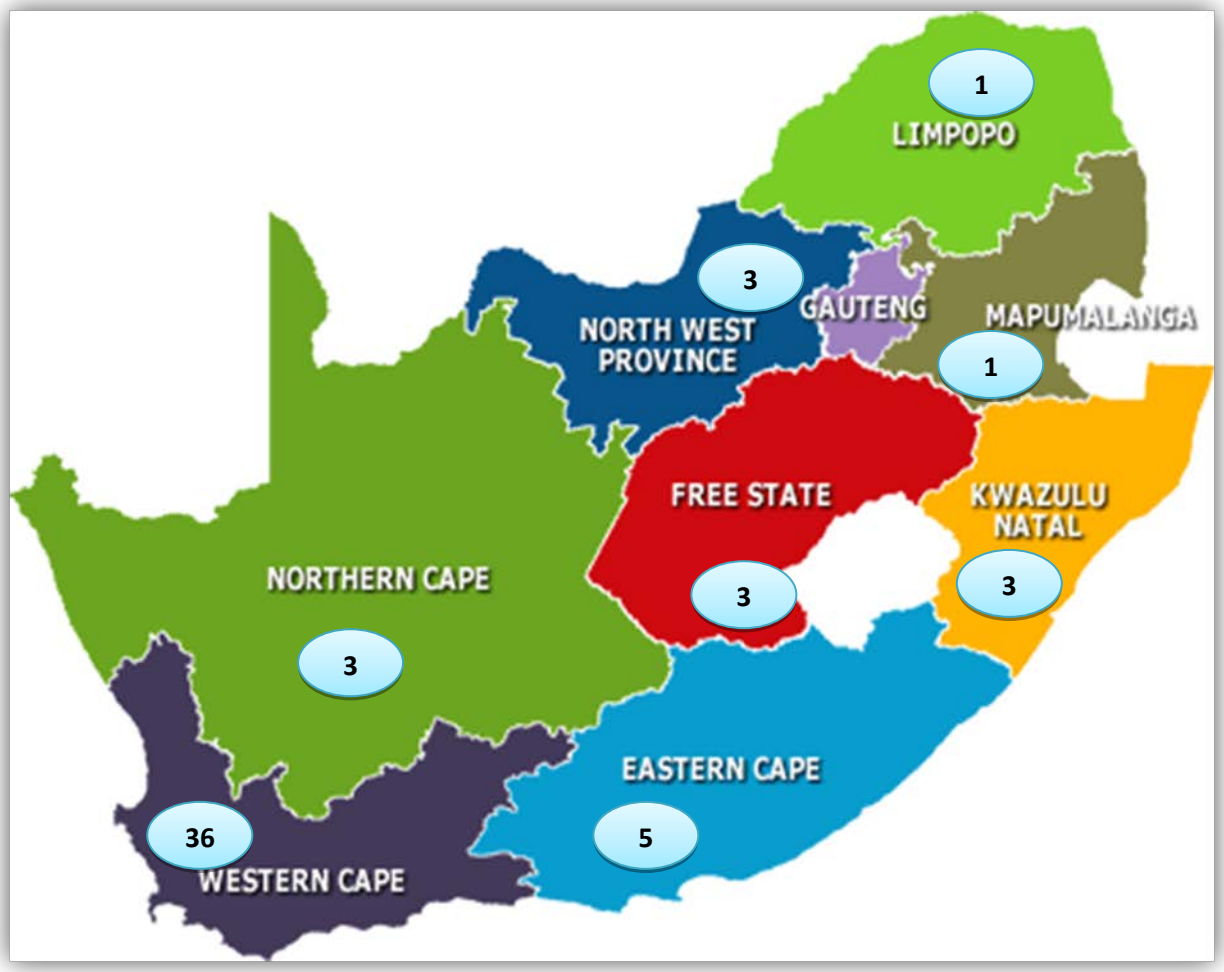


Figure 2.2: Map of SA illustrating geographical region and number of honey samples acquired from each region.

Table 2.1: South African honey samples collected in various geographical regions in 2011.

REFERENCE CODE	SCIENTIFIC NAME (PERCEIVED BOTANICAL SOURCE)	LOCALITY	PROVINCE	SOURCE DATE
1–(CITYMIXA/EC)	*Eucalyptus* spp., *Trichilia emetica, Erythina* spp. (Inner city A mix)	East London	E. Cape	Apr-11
2-(CITYMIXB/EC)	*Callistemon viminalis, Eugenia* spp., *Eucalyptus* spp., *Jacaranda* spp., *Ocimum basilicum, Thymus* spp., *Lavandula* spp., *Leguminosae* spp., *Juglans cinerea, Pisum sativum, vitis* spp., *Persea* spp. and others, (Inner city B mix)	East London	E. Cape	Apr-11
3-(CITYMIXC/EC)	*Acacia karroo, Allophylus decipiens, Allophylus natalensis, Apodytes dimidiata, Asparagus* spp., *Buddleja auriculata, Buddleja saligna, Carissa bispinosa, Dombeya rotundifolia, Dovyalis caffra, Dovyalis longispina, Dovyalis zeyheri, Ehertia rigida, Euclea natalensis, Grewia occidentalis, Gymnosporia mossambicensis, Pavetta revoluta, Plumbago auriculata, Ptaeroxylon obliquum, Putterlickia pyracantha, Putterlickia verrucosa, Rhus glauca, Rhus incisa, Rhus laevigata, Syzygium cordatum, Tecomaria capensis*	Strand	W. Cape	Apr-11

REFERENCE CODE	SCIENTIFIC NAME (PERCEIVED BOTANICAL SOURCE)	LOCALITY	PROVINCE	SOURCE DATE
4-(CITYMIX/EC)	Unknown	Strand	W. Cape	Apr-11
5-(CITYMIXTUART/WC)	*Eucalyptus gomphocephala and* Unknown (Tuart gum and City Mix)	Strand	W. Cape	Apr-11
6-(MANGO/WC)	*Mangifera indica* (Mango)	Dutoitskloof	W. Cape	Apr-11
7–(FYNBOS/WC)	*Ericas* spp. (Fynbos)	Bainskloof	W. Cape	Apr-11
8–(ECFYNBOS/WC)	*Eucalyptus cladocalyx* and *Erica* spp. (Sugar Gum and Fynbos)	Llandudno	W. Cape	Jan-11
9–(STANDVELD/WC)	(Strandveld)	Hermanus	W. Cape	Feb-11
10-(BUCHU/WC)	*Agathosma* spp.	Hopefield	W. Cape	Feb-11
11-(STANDVELD/WC)	Unknown	Heidelburg	W. Cape	Jan-11
12–(BUSHVELD/KZN)	(Coastal Sandy Bushveld)	Maputaland	KZN	Jan-11
13–(BUFFALOTHORN/NC)	*Ziziphus mucronata* (Buffalo Thorn)	Northern Cape	N. Cape	Mar-11
14–(HOOKTHORN/NC)	*Acacia mellifera* (Hookthorn)	Northern Cape	N. Cape	Mar-11
15–(ONION/WC)	*Allium cepa* (Onion)	Oudtshoorn	W. Cape	Feb-11

REFERENCE CODE	SCIENTIFIC NAME (PERCEIVED BOTANICAL SOURCE)	LOCALITY	PROVINCE	SOURCE DATE
16-(FYNBOS/WC)	*Erica* spp. (Fynbos)	Mossel Bay	W. Cape	Feb-11
17-(AKMS/FS)	Acacia karroo and medicago sativa (Soetdoring and Lucerne)	Glen	Free State	Feb-11
18–(MIXEDGUM/FS)	(Mixed gums)	Bainsvlei	Free State	Mar-11
19–(CITYMIX/FS)	(City mix)	Rayton	Free State	Mar-11
20–(FORRESTREDGUM/WC)	*Eucalyptus tereticornis* (Forrest red gum)	Mariendahl Stellenbosch	W. Cape	Jan-11
21–(SUGARGUM/WC)	*Eucalyptus cladocalyx* (Sugar Gum)	Kromme Rhee	W. Cape	Mar-11
22-(FYNBOS/WC)	*Erica* spp.	Lourensford Somerset west	W. Cape	Mar-11
24–(STRANDVELD/WC)	(Strandveld wild flowers)	Still Bay	W. Cape	Apr-11
25–(BUCFYN/WC)	*Agathosma* spp./ *Erica* spp. (Fynbos\Buchu)	Hermanus	W. Cape	Mar-11
26–(FYNBOS/WC)	*Erica ericoides* (Fynbos)	Cape Point	W. Cape	Mar-11
27–(AEF/WC)	*Agathosma* spp./*Eucalyptus ficifolia* (Buchu and Red Flowering Gum)	Kogelburg	W. Cape	Jan-11
31–(MACADAMIA/WC)	*Macadamia integrifolia* (Macadamia)	Herbertsdal	W. Cape	Jan-11
32-(FYNBOS/WC)	*Erica* spp.	Herbertsdal	W. Cape	Jan-11
33-(CITRUS/WC)	*Citrus* spp.	Citrusdal	W. Cape	Mar-11
34-(ONION/FYNBOS)	*Allium Cepa/Erica* spp.	Ceres	W. Cape	Mar-11
35-(FYNBOSEC/WC)	*Eucalyptus conferruminata/Erica* spp. (Spider Gum and Fynbos)	Pearly Beach	W. Cape	Mar-11
36-(EUCLADFIC/WC)	*Eucalyptus cladocalyx/Eucalyptus ficifolia*	Somerset West	W. Cape	Mar-11
37–(FYNBOS/WC)	*Erica* spp. (Fynbos)	Stanford	W. Cape	Mar-11
38–(WASBESSFYNBOS/WC)	*Morella cordifolia/Erica* spp. (Wasbessie and fynbos)	Albertina	W. Cape	Mar-11
39-(SUGUARGUM/WC)	*Eucalyptus cladocalyx*	Moorreesburg	W. Cape	Feb-11

REFERENCE CODE	SCIENTIFIC NAME (PERCEIVED BOTANICAL SOURCE)	LOCALITY	PROVINCE	SOURCE DATE
40–(FYNBOSGUARRI/WC)	*Erica* spp. and *Euclea racemosa* (Fynbos, guarri)	Stanford	W. Cape	Jan-11
41–(INDIGENOUS/WC)	*Podocarpus latifolius, Dalbergia melanoxylon, Acacia* spp.*,Pyrus* spp.*, Eucalyptus tereticornis, Acacia karroo, Melaleuca* spp.*, Acacia erioloba, Aloe* spp.*, pelargonium* spp.*, Cotyledon orbiculata, Aponogeton distachyos, Nymphaea* spp.*, Hypoxis hemerocallidea* (indigenous and others)	Oudtshoorn	W. Cape	Feb-11
43–(KAROOVELD/EC)	(Karoo veld)	Aberdeen	E. Cape	Mar-11
44–(STRANDVELD/WC)	(Strandveld)	Albertina	W. Cape	Mar-11
45–(FYNBOS/WC)	*Erica* spp. (Fynbos)	Still Bay	W. Cape	Mar-11
46-(KARRIGUM/WC)	*Eucalyptus diversicolor*	Knysna	W. Cape	Mar-11
47–(SALIGNAGUM/KZN)	*Eucalyptus grandis* (Saligna Gum)	Howick	KZN	Mar-11
48–(BRE/EC)	(Bushveld, riverine forest, euphorbias)	Baviaanskloof	E. Cape	Feb-11
49-(CITRUS/EC)	*Citrus* spp. (Citrus)	Sundays River Valley	E. Cape	Feb-11
50-(HOOKTHORN/WC)	Acacia mellifera	Prieska	W. Cape	Mar-11
51-(URBANFORAGE/WC)	(Urban Forage)	Liesbeeck River	W. Cape	Mar-11
52-(SUBURBANGARDEN/WC)	(Suburban gardens)	Constantia	W. Cape	Mar-11
53-(LITCHI/MP)	*Litchi chinensis* (Litchi)	White River	Mpumalanga	Jan-11
154-(ONION/WC)	*Allium cepa*	Mossel Bay	W. Cape	Jan-11

REFERENCE CODE	SCIENTIFIC NAME (PERCEIVED BOTANICAL SOURCE)	LOCALITY	PROVINCE	SOURCE DATE
156-(BUFFALOTHORN/NC)	*Ziziphus mucronata* (Buffalo Thorn)	Douglas	N. Cape	Jan-11
158-(BUFFALOTHORN/NW)	*Ziziphus mucronata* (Buffalo Thorn)	Christiana	North West	Jan-11
159-(BUFFALOTHORN/NW)	*Ziziphus mucronata* (Buffalo Thorn)	Potchefstroom	North West	Jan-11
160-(BOEKENHOUT/LIM)	*Faurea saligna* (Boekenhout)	Naboomspruit	Limpopo	Jan-11
162-(SALIGNAGUM/KZN)	*Eucalyptus grandis* (Saligna Gum)	Harding	KZN	Sep-11
167-(SUNFLOWER/NW)	(Sunflower)	Ventersdorp	North West	Feb-11
54-(MANUKA/NZ)	*Leptospermum scoparium* (Manuka honey) Control	Coromandel	New Zealand	Feb-11
55-(MANUKA/NZ)	*Leptospermum scoparium* (Manuka honey) Control	Kailaia	New Zealand	Feb-11
56-(MANUKA/NZ)	*Leptospermum scoparium* (Manuka honey) Control	Ohakune	New Zealand	Feb-11

Reference code: Sample number – (Common name or scientific name/ Province abbreviated); WC – Western Cape; NC- Northern Cape; EC- Eastern Cape; KZN- Kwa-Zulu Natal; FS- Free State; MP- Mpumalanga; LIM – Limpopo; NZ – New Zealand

Table 2.2: Honey samples collected prior to 2011.

REFERENCE CODE	SCIENTIFIC NAME (PERCEIVED BOTANICAL SOURCE)	LOCALITY	PROVINCE	SOURCE DATE
60-(YELLOWBOX/WC)	*Eucalyptus melliodora* (Yellow Box)	Darling	W. Cape	Jul-08
61-(STRANDVELD/WC)	Unknown (Strandveld)	Hopefield	W. Cape	Jul-08
62-(SUGARGUM/WC)	*Eucalyptus cladocalyx* (Sugar Gum)	Paarl	W. Cape	Jul-08
64-(CITRUS/WC)	*Citrus* spp. (Citrus)	Citrusdal	W. Cape	Jul-08
69-(OLIVEFYN/SC)	*Olea* spp. *and Erica* spp. (Wild olive and Fynbos)	Southern Cape	S. Cape	Jan-09
80-(FORESTREDGUM/WC)	*Eucalyptus tereticornis* (Forest Red Gum)	Elsenburg	W. Cape	Sep-08
86-(LITCHI/MP)	*Litchi chinensis* (Litchi)	White River	Mpumalanga	Jul-08
90-(FYNBOS/WC)	*Erica* spp. (Fynbos)	Bainskloof	W. Cape	Jul-08
94-(HOOKTHORN/NC)	*Acacia mellifera* (Hookthorn)	Douglas	N. Cape	Jul-08
106-(ONION/WC)	*Allium cepa* (Onion)	Ceres	W. Cape	Apr-08
109-(FYNBOS/WC)	*Erica* spp. (Fynbos)	Mossel Bay	W. Cape	Jul-08
116-(FYNBOS/SC)	*Erica* spp. (Fynbos)	S Cape	S. Cape	Jul-08
117-(WILDFLOWERS/GP)	(Wild flowers)	Highveld	Gauteng	Jul-08
119-(BUFFALOTHORN/NW)	*Ziziphus mucronata* (Buffalo Thorn)	Christiana	North West	Jul-08
123-(SALIGNAGUM/KZN)	*Eucalyptus grandis* (Saligna Gum)	Harding	KZN	Mar-08
135-(FYNBOS/WC)	*Erica* spp. (Fynbos)	Stanford	W. Cape	Jul-07
139-(BLUEBUSH/NW)	*Diospyros lycioides* (Blue Bush)	Christiana	North West	Oct-10
142-BUFFALOTHORN/NW)	*Ziziphus mucronata* (Buffalo Thorn)	Christiana	North West	2010
144-(FYNBOS/WC)	*Erica* spp. (Fynbos)	Mossel Bay	W. Cape	Oct/Nov/2010

REFERENCE CODE	SCIENTIFIC NAME (PERCEIVED BOTANICAL SOURCE)	LOCALITY	PROVINCE	SOURCE DATE
145-(COSMOS/NP)	*Cosmos bipinnatus*	NP	NP	2010
146-(REDSYRING/LIM)	*Burkea africana* (Red syringe)	Waterberg	Limpopo	Jan-09
148-(ALOE/NP)	*Greatheadii var davyana* (Aloe)	NP	NP	Aug-09
149-(HOOKTHORN/NP)	*Acacia mellifera* (Hookthorn)	NP	NP	Oct-10
150-(COMBRETUM/GP)	*Combretum* and others (Wild Flora)	North Pretoria	Gauteng	Oct/Nov 2010
155-(CATTHORN/EC)	*Scutia myrinta* (Cat Thorn)	Port Alfred	E. Cape	Dec-2010
96-(MANUKA/NZ)	*Leptospermum scoparium* (Manuka honey) Control	Unknown	New Zealand	Apr-2008
97-(MANUKA/NZ)	*Leptospermum scoparium* (Manuka honey) Control	Unknown	New Zealand	Jul-2008
147-(MANUKA/NZ)	*Leptospermum scoparium* (Manuka honey) Control	Unknown	New Zealand	Unknown

Reference code: Sample number – (Common name or scientific name/ Province abbreviated); WC – Western Cape; NC- Northern Cape; EC- Eastern Cape; KZN- Kwa-Zulu Natal; FS- Free State; MP- Mpumalanga; LIM – Limpopo; NZ – New Zealand

2.2.3 Test pathogen strains

A variety of pathogens most commonly responsible for wound microbial colonisation and proliferation were selected to test the antimicrobial efficacy of the acquired honey samples.

The pathogens included;

- Six Gram-positive strains: *Staphylococcus aureus* (laboratory - ATCC 25923), *S. aureus,* (clinical – ATCC 6438300), MRSA (laboratory – ATCC 43300), MRSA (clinical – ATCC 43300), methicillin and gentamicin resistant *S. aureus* (laboratory – ATCC 33592), *Staphylococcus epidermidis* (laboratory – ATCC 2223).
- One Gram-negative strain: *Pseudomonas aeruginosa* (laboratory – ATCC 9027).
- Two yeast strains: *Candida albicans* (laboratory – ATCC 10231), *Candida albicans* (clinical – CA9B).

Stock cultures for ATCC strains were obtained from Davies Diagnostics and clinical strains were obtained from Dr. M. Patel (Department of Oral Microbiology, University of the Witwatersrand). All pathogens were grown in Tryptone Soya Broth (TSB). The stock cultures were refrigerated at a temperature of -20 °C. A waiver was obtained for the use of clinical strains (Appendix B).

2.2.4 Preparation of media:

Tryptone Soya Agar (TSA) was prepared by suspending 30 g of Tryptone Soya powder in 750 mL of distilled water. Tryptone Soya Broth (TSB) was prepared by suspending 40 g of Tryptone Soya powder in 1 L of distilled water. The agar and broth was subsequently autoclaved at 121 °C for 15 minutes. Sterility of the agar and broth were ensured by incubating the broth and agar to 37 °C for 24 hours before use and inspecting visually for contamination.

2.2.5 Evaluation of the mean inhibitory concentration

As per National Committee for Clinical Laboratory Standards (NCCLS) guidelines (2003), three to five isolated colonies were selected from a TSA agar plate culture. The growth was then transferred to a test tube containing 5 mL of TSB. The broth containing the culture was then incubated at 37 °C for 24 hours for bacterial pathogens and 48 hours for yeasts. Purity

of cultures was ensured by streaking onto an agar plate and incubating at 37 °C for 24 hours for bacterial species and 48 hours for yeasts.

The turbidity of the broth culture was visually adjusted with TSB to achieve a 0.5 McFarland Standard. The 0.5 McFarland standard suspension was then diluted to a 1:100 in sterile TSB which resulted in a concentration of approximately $1x10^7$ colony forming units (CFU)/mL. Figure 2.3 describes the inoculation of honey-agar plates with the respective pathogens. A multipoint inoculator, multipointelite™ was utilised to automatically deliver the test pathogens to the honey-agar plate. The multipoint inoculator delivers approximately 1 – 2 µL on the agar surface.

Antimicrobial activity was established by determining the MIC utilising the agar dilution method. Agar dilution involves the incorporation of different concentrations of the antimicrobial agent (honey) into a nutrient agar medium and the subsequent inoculation. The agar was contained in a water bath at 55 °C to ensure that it did not solidify and that its contained viscosity was desirable. The MIC was defined as the lowest concentration of honey required for inhibiting visible growth of the pathogens being investigated. Honey dilutions ($^v/_v$) of 50.00%, 25.00%, 12.50%, 6.25% and 3.13% were analysed. Honey dilutions were added to molten TSA medium that was allowed to equilibrate in a water bath at 55 °C.

The agar and honey at various concentrations (50.00%, 25.00%, 12.50%, 6.25% and 3.13%) ($^v/_v$) were pipetted into sterile test tubes and subsequently vortex mixed to ensure a homogenous distribution of the mixture. The agar and honey solution was then poured into petri dishes to achieve a depth of 3 mm (20 mL per plate). This process should occur swiftly to prevent cooling, solidification before settling and the formation of bubbles. Cultures were inoculated onto the surface of the agar utilising a multipointelite innoculator as described in Figures 2.3. One millilitre of the 1:100 cultures was pipetted into three wells of the multipointelite innoculator. After inoculation, the plates were allowed to stand for 30 minutes to ensure that the inoculum spots were absorbed into the agar. After inoculation, the agar-honey dishes were incubated for 24 hours at 37 °C for Gram-positive and Gram-negative bacteria, subsequently removed with the results being read and then replaced back into the incubator. The plates were further incubated for an additional 24 hours to accommodate and investigate growth of the yeast species. Growth control plates of TSA were inoculated first and last to ensure the appropriate growth of test micro-organisms. To ensure accuracy and consistency, all MIC's were performed in triplicate (N=3). Results were reported in accordance with the presence or absence of microbial growth on the agar plates. All MIC values were expressed in % ($^v/_v$).

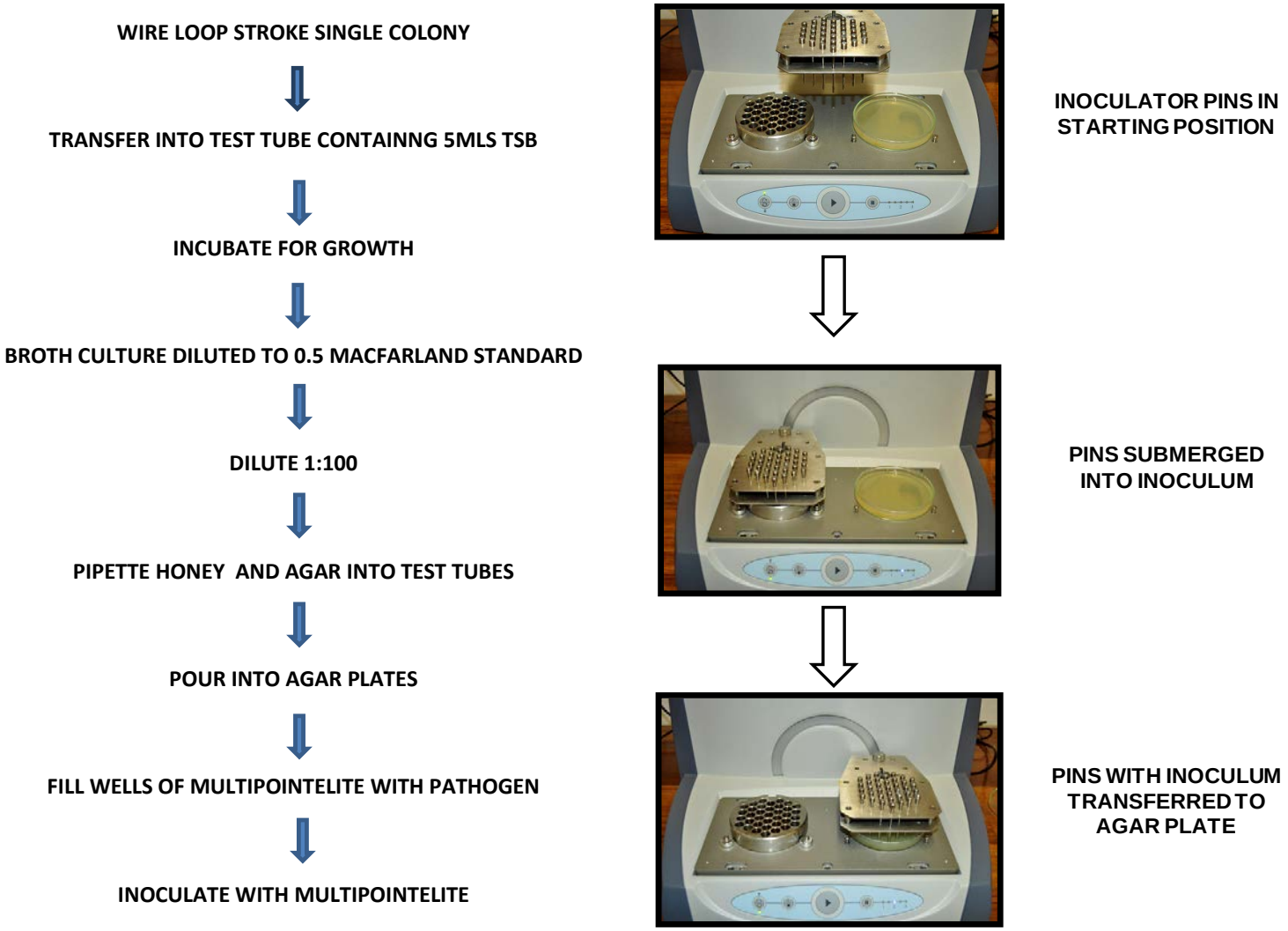


Figure 2.3: Flow Diagram illustrating the steps for inoculation of honey-agar plates and the multipointelite inoculator inoculating honey-agar plates.

2.2.6 Controls

2.2.6.1 Artificial honey

An artificial honey solution (39.00% $^{w}/_{v}$ d-fructose, 31.00% $^{w}/_{v}$ d-glucose, 8.00% $^{w}/_{v}$ maltose, 3.00 % $^{w}/_{v}$ sucrose and 19.00% $^{w}/_{v}$ water) with a sugar content similar to honey was included in the assays as a control to determine antimicrobial effects due to sugar. Sugar is known to possess some antimicrobial properties (Molan, 2001; Basson and Grobler 2008). The artificial honey solution was autoclaved at 121 °C for 15 minutes and subjected to the agar dilution method at concentrations of 50.00%, 25.00%, 12.50%, 6.25% and 3.13% to mimic honey concentrations.

2.2.6.2 Manuka honey

This honey was utilised as a comparative control with which to compare SA honeys. Manuka honey is derived from the *Leptospermum scoparium* floral source, in New Zealand. Six samples were obtained where three of which were collected in 2011, two were collected in 2008 and one of which the collection date is unknown.

2.2.6.3 Phenol

A 20% ($^{w}/_{v}$) phenol solution prepared by dissolving 20 g of phenol powder in 100 mL of sterile water, was utilised as a positive control. Phenol and agar were mixed to produce a 20% phenol control and subsequently inoculated as honey agar plates. A blank TSA plate was utilised as a growth control. This was used between every honey-agar plate.

2.2.6.4 Ciprofloxacin

The broad-spectrum conventional antibiotic, ciprofloxacin, was also used as a control in this study.

2.3 Results and discussion

2.3.1 Mean inhibitory concentration

The mean MIC sensitivity (% inhibition) of each honey collected in various geographical regions for the year 2011 is depicted in Table 2.3.

Table 2.3: Minimum inhibitory concentration (% inhibition) of samples collected in 2011.

REFERENCE CODE	*S. aureus* ATCC 25923	*S. aureus* clinical	*S. aureus* (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC 2223	*P. aeruginosa* ATCC 9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
1-(CITYMIXA/EC)	12.50	12.50	12.50	12.50	12.50	12.50	6.25	50.00	25.00	17.36±13.18
2-(CITYMIXB/EC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
3-(CITYMIXB/EC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
4-(CITYMIX/WC)	6.25	6.25	6.25	6.25	12.50	6.25	6.25	50.00	25.00	13.89±14.91
5-(CITYMIXTUART/WC)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00 ±0.00
6-(MANGO/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
7-(FYNBOS/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
8-(ECFYNBOS/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02

REFERENCE CODE	***S. aureus* ATCC 25923**	***S. aureus* clinical**	***S. aureus* (MRSA) 43300**	***S. aureus* (MRSA) clinical**	***S. aureus* (M & G)* ATCC 33592**	***S. epidermidis* ATCC 2223**	***P. aeruginosa* ATCC 9027**	***C. albicans* ATCC 10231**	***C. albicans* clinical**	**MEAN MIC**
9-(STRANDVELD/WC)	25.00	25.00	25.00	25.00	25.00	25.00	6.25	50.00	50.00	28.47±13.66
10-(BUCHU/WC)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
11-(STRANDVELD/WC)	50.00	50.00	50.00	50.00	50.00	25.00	25.00	50.00	50.00	44.44±11.02
12-(BUSHVELD/KZN)	12.50	12.50	12.50	12.50	12.50	12.50	6.25	25.00	25.00	14.58±6.25
13-(BUFFALOTHORN/NC)	25.00	25.00	25.00	25.00	25.00	25.00	6.25	25.00	50.00	25.69±11.02
14-(HOOKTHORN/NC)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
15-(ONION/WC)	12.50	12.50	12.50	12.50	12.50	12.50	12.50	25.00	25.00	15.28±5.51
16-(FYNBOS/WC)	6.25	6.25	6.25	6.25	6.25	6.25	6.25	25.00	25.00	10.42±8.27
17-(AKMS/FS)	12.50	12.50	12.50	12.50	12.50	12.50	6.25	25.00	25.00	14.58±6.25

REFERENCE CODE	*S. aureus* ATCC 25923	*S. aureus* clinical	*S. aureus* (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC 2223	*P. aeruginosa* ATCC 9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
18-(MIXEDGUM/FS)	6.25	6.25	6.25	6.25	6.25	6.25	6.25	50.00	50.00	15.97±19.29
19-(CITYMIX/FS)	6.25	6.25	6.25	6.25	6.25	6.25	6.25	50.00	6.25	11.11±14.58
20-(FORRESTREDGUM/WC)	6.25	12.5	12.5	6.25	6.25	6.25	6.25	50.00	50.00	17.36±18.69
21-(SUGARGUM/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
22—(FYNBOS/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
24-(STRANDVELD/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00 ±0.00
25–(BUCFYN/WC)	25.00	25.00	25.00	25.00	25.00	12.50	12.50	50.00	25.00	25.00±10.83
26-(FYNBOS/WC)	12.50	12.50	12.50	12.50	12.50	12.50	6.25	50.00	50.00	20.14±17.05

REFERENCE CODE	*S. aureus* ATCC 25923	*S. aureus* clinical	*S. aureus* (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC 2223	*P. aeruginosa* ATCC 9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
27-(AEF/WC)	25.00	25.00	25.00	25.00	25.00	12.50	6.25	25.00	12.50	20.14±7.51
31-(MACADAMIA/WC)	25.00	25.00	25.00	25.00	25.00	12.50	12.50	50.00	50.00	27.78±13.66
32-(FYNBOS/WC)	50.00	50.00	50.00	12.50	50.00	25.00	25.00	50.00	50.00	40.28±15.02
33-(CITRUS/WC)	50.00	50.00	25.00	25.00	50.00	25.00	25.00	50.00	50.00	38.89±13.18
34-(ONION/FYNBOS)	50.00	50.00	50.00	50.00	50.00	50.00	25.00	50.00	50.00	47.22±8.33
35-(FYNBOSEC/WC)	25.00	25.00	25.00	25.00	25.00	25.00	12.50	50.00	25.00	26.39±9.77
36-(EUCLADFICI/WC)	12.50	12.50	12.50	12.50	25.00	12.50	6.25	25.00	25.00	15.97±7.06
37-(FYNBOS/WC)	12.50	25.00	12.50	12.50	25.00	25.00	25.00	50.00	50.00	26.39±14.58
38-(WASBESSFYNBOS/C)	12.50	12.50	12.50	12.50	12.50	12.50	6.25	50.00	50.00	20.14±17.05

REFERENCE CODE	*S. aureus* ATCC 25923	*S. aureus* clinical	*S. aureus* (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC 2223	*P. aeruginosa* ATCC 9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
39-(SUGARGUM/WC)	50.00	50.00	50.00	50.00	50.00	25.00	50.00	50.00	50.00	47.22±8.33
40-(FYNBOSGUARRI/WC)	25.00	25.00	25.00	25.00	50.00	25.00	12.50	50.00	50.00	31.94±14.13
41-(INDIGENOUS/WC)	12.50	12.50	6.25	6.25	12.50	12.50	6.25	25.00	25.00	13.19±7.29
43-(KAROOVELD/EC)	12.50	12.50	12.50	25.00	12.50	12.50	6.25	50.00	50.00	21.53 ±16.86
44-(STRANDVELD/WC)	12.50	25.00	12.50	12.50	25.00	12.50	6.25	50.00	50.00	22.92±16.54
45-(FYNBOS/WC)	12.50	12.50	12.50	12.50	12.50	12.50	6.25	50.00	50.00	20.14±17.05
46-(KARRIGUM/WC)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
47-(SALIGNAGUM/KZN)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
48-(BRE/EC)	25.00	25.00	25.00	25.00	25.00	25.00	12.50	50.00	50.00	29.17±12.50

REFERENCE CODE	*S. aureus* ATCC 25923	*S. aureus* clinical	*S. aureus* (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC 2223	*P. aeruginosa* ATCC 9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
49-(CITRUS/EC)	50.00	50.00	50.00	50.00	50.00	50.00	6.25	50.00	50.00	45.14±14.58
50-(HOOKTHORN/WC)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
51-(URBANFORAGE/WC)	12.50	6.25	6.25	6.25	12.50	6.25	6.25	50.00	50.00	17.36±18.69
52-(SUBURBANGARDEN/WC)	6.25	12.50	6.25	6.25	12.50	6.25	6.25	50.00	25.00	14.58±14.66
53-(LITCHI/MP)	50.00	50.00	50.00	50.00	50.00	50.00	6.25	50.00	50.00	45.14±14.58
154-(ONION/WC)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
156-(BUFFALOTHORN/NC)	25.00	25.00	25.00	25.00	25.00	25.00	12.50	50.00	50.00	29.17±12.5
158-(BUFFALOTHORN/NW)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02

REFERENCE CODE	*S. aureus* ATCC 25923	*S. aureus* clinical	*S. aureus* (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC 2223	*P. aeruginosa* ATCC 9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
159-(BUFFALOTHORN/NW)	25.00	25.00	25.00	25.00	25.00	25.00	12.50	50.00	50.00	29.17±12.50
160-(BOEKENHOUT/LIM)	25.00	25.00	25.00	25.00	25.00	6.25	6.25	50.00	50.00	26.39±15.55
162-(SALIGNAGUM/KZN)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±12.50
167-(SUNFLOWER/NW)	25.00	25.00	25.00	25.00	25.00	25.00	12.50	50.00	50.00	29.17D±10.18
Mean of pathogens	26.36 ±15.39	26.93 ±15.02	25.80 ±15.17	25.23±14.92	27.73±15.06	23.63 ±14.37	18.75 ±15.12	45.91 ±9.33	43.07 ±12.31	

(M&G)* = methicillin and gentamicin resistant *S. aureus* (laboratory – ATCC 33592)

Table 2.4: Minimum inhibitory concentration (% inhibition) of controls.

CONTROL	*S. aureus* ATCC 25923	*S. aureus* clinical	S. aureus (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC 2223	*P. aeruginosa* ATCC 9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
54-(MANUKA/NZ)	12.50	12.50	12.50	12.50	12.50	25.00	12.50	25.00	12.50	15.28±5.51
55-(MANUKA/NZ)	6.25	12.50	6.25	6.25	25.00	12.50	12.50	50.00	50.00	20.17±17.89
56-(MANUKA/NZ)	50.00	50.00	50.00	25.00	50.00	25.00	25.00	50.00	50.00	41.67±12.50
Artificial Honey	50.00	50.00	50.00	50.00	50.00	25.00	25.00	50.00	50.00	44.44±11.02
Ciprofloxacin (µg/ml)	0.078	0.078	0.078	1.25	0.078	0.30	0.25	N/A	N/A	N/A
Amphotericin B	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.25	1.25	N/A

N/A Not applicable

The MIC against all pathogens ranged from 10.42-50.00%, with sample 16-(FYNBOS/WC) displaying the most broad-spectrum activity. At a concentration of 50.00%, the growth against all nine test pathogens was completely inhibited by all honey samples. This was consistent with artificial honey thus it can be postulated that the antimicrobial activity of these samples can be attributed to hyper-osmolar effects as the carbohydrate concentration has a pivotal effect in the antimicrobial activity of honeys above 25%. Hyper-osmolarity prevents the growth of bacteria and promotes wound healing (Basson and Grobler, 2008). It was observed that *P. aeruginosa* was the most sensitive strain, as the mean MIC for all honeys tested against *P. aeruginosa* was 18.75±15.12%. The mean MIC sensitivity of each honey collected in 2011 to the respective pathogen strains are reported in Figure 2.4.

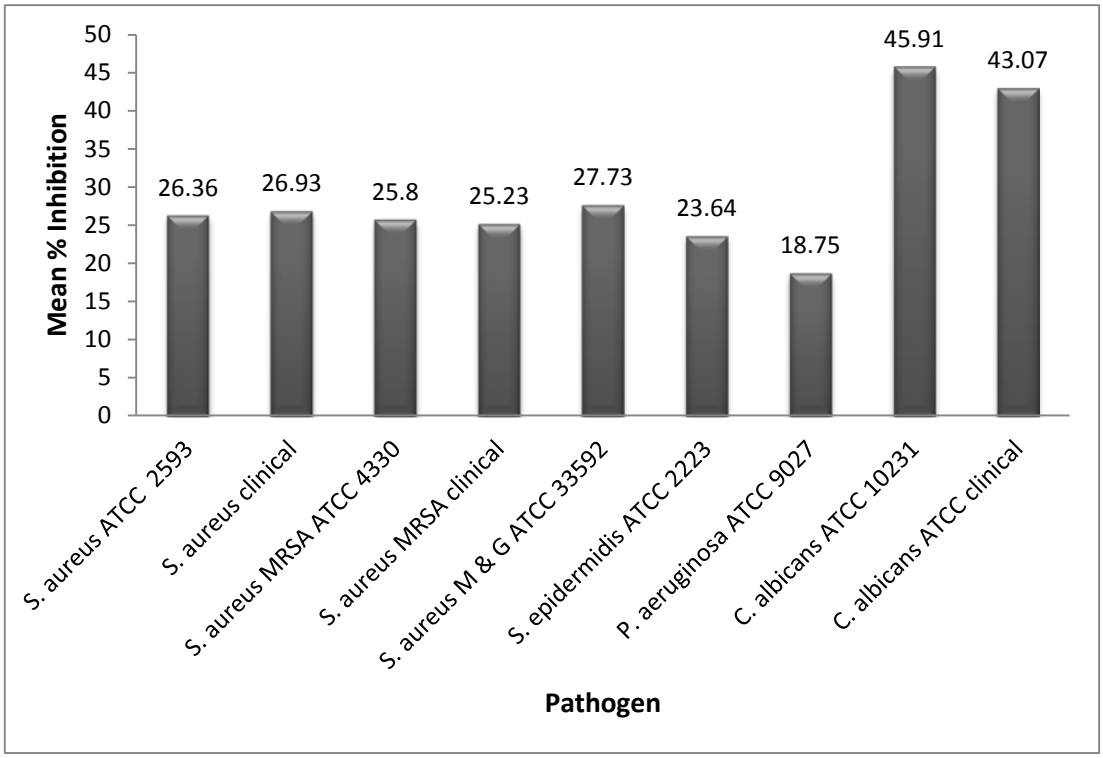


Figure 2.4: Mean MIC results for all pathogens tested against SA honey samples.

Manuka honey and artificial honey were utilised as controls (Table 2.4). The antimicrobial activity of these samples ranged from 15.28±5.51-41.67±12.50% (N=3), with the average activity of these three samples being 25.69±14.04%. Any sample with an MIC ≤ 25.69% was considered desirable and good. Currently in Australia and New Zealand, Medihoney® and Manuka honey are marketed as therapeutic honeys suitable for use in ulcers, infected wounds and burns. Research has indicated that both these honeys, derived from the *Leptospermum* spp., have considerable antimicrobial activity against various wound organisms as well as antibiotic resistant bacteria (Brady et al., 2004). The Manuka honey

from Coromandel, New Zealand, (54-MANUKA/NZ) exhibited the most desirable antimicrobial activity of the three samples tested. Samples tested including; {1-(CITYMIXA/EC), 4-(CITYMIX/WC), 12–(BUSHVELD/KZN), 15-(ONION/WC), 16–(FYNBOS/WC), 17–(AKMS/FS), 18–(MIXEDGUM/FS), 19–(CITYMIX/FS), 20–(FORRESTREDGUM/WC), 24–(STRANDVELD/WC), 25–(BUCFYN/WC), 26–(FYNBOS/WC), 27–(AEF/WC), 36–(EUCLADFICI/WC), 38–(WASBESSFYNBOS/WC), 41–(INDIGENOUS/WC), 43–(KAROOVELD/EC), 44–(STRANDVELD/WC), 45–(FYNBOS/WC), 51–(URBANFORAGE/WC) and 52-(SUBURBANGARDEN/WC)} had a greater antimicrobial activity (lower % inhibition) than when compared to the mean Manuka honey samples. Honey sample (13–(BUFFALOTHORN/NC) displayed antimicrobial activity equivalent to that of the mean Manuka honey activity. Honey sample 16–(FYNBOS/WC) displayed the greatest antimicrobial activity with a mean MIC of 10.42±8.27%. The Manuka honey sample 54-(MANUKA/NZ) with the best antimicrobial activity observed for the controls had a mean MIC of 15.28%. Seven honey samples including; {4-(CITYMIXA/EC), 12-(BUSHVELD)/KZN, 16-(FYNBOS/WC), 17-(AKMS/FS), 19-(CITYMIX/FS), 41-(INDIGENOUS/WC) and 52-SUBURBANGARDEN/WC} had comparatively superior activity, having MICs lower than 15.28% observed for the Manuka honey sample exhibiting the best antimicrobial activity.

Honey samples 1-(CITYMIXA/EC), 4-(CITYMIX/WC), 9-(STRANDVELD/WC), 12-(BUSHVELD/KZN), 13-(BUFFALOTHORN/KZN), 16-(FYNBOS/WC), 17 –(AKMS/FS), 18-(MIXEDGUM/FS), 19-(CITYMIX/FS), 20-(FORRESTREDGUM/WC), 26-(FYNBOS/WC), 27-(AEF/WC), 36-(EUCLADFICI/WC), 38-(WASBESSFYN/WC), 41-(INDIGENOUS/WC), 43-(KAROOVELD/EC), 44-(STRANDVELD/WC), 45-(FYNBOS/WC), 49-(CITRUS/EC), 51-(URBANFORAGE/WC), 52-(SUBURBANGARDEN/WC), 53-(LITCHI/MP), and 160-(BOEKENHOUT/LIM) demonstrated considerable efficacy (MIC = 6.25%) against *P. aeruginosa*. This holds promise for SA honey as an alternative antimicrobial because of the severity of *Pseudomonas*-related infections particularly in immunocompromised patients (Speert, 2002). This Gram-negative organism is considered to be one of the most serious clinical complications in burn patients resulting in systemic infection (Nasser et al., 2003). *Pseudomonas* spp. are also commonly associated with nosocomial infections (Khoo et al., 2010). This pathogen is often difficult to eradicate as it develops antibiotic resistance (Lambert, 2002). Mullai and Menon (2005) assessed the antimicrobial activity of Manuka honey (Australia), Heather honey (United Kingdom) and Khadikraft honey (India), against 152 *P. aeruginosa* strains, which exhibited an MIC ranging from 10.00% - 20.00% for all honeys tested. Mandal and co-workers (2010), reported an MIC value of 3.50% ($^{v}/_{v}$) for *Pseudomonas* isolates tested against a honey sample harvested from an *indica* hive in India. Cooper (1999) also reported that Manuka honey produced an MIC ranging from 5.50-8.70%

for *P. aeruginosa* strains from infected wounds (Cooper, 1999). Similarly, Cooper and co-workers (2002) reported that Manuka honey had an MIC value of 7.50% against clinical strains of *P. aeruginosa* from infected burn wounds (Cooper et al., 2002). An *in vivo* investigation by Khoo and co-workers (2010), revealed that *P. aeruginosa* was inhibited by Tualang honey at a concentration of 0.1 mL/cm^2. In this study, it was observed that 41.82% of samples tested had an MIC value of 6.25% against *P. aeruginosa.* SA honey thus demonstrates the potential to heal potentially problematic burn wounds in patients that may lead to systemic sepsis. This is because it has been anticipated that honeys which produce an MIC between 10.00% to 20.00% should be effective in eradicating *Pseudomonas* infections (Mullai and Menon, 2005).

S. epidermidis has been commonly identified in skin wounds and is considered to be part of the natural skin flora. This commensal can be pathogenic and has also developed antibiotic resistance (Basualdo et al., 2007; Chusri et al., 2012). *S. epidermidis* infections are usually acquired nosocomially (Raad et al., 1998). This study confirmed that *S. epidermidis* was substantially inhibited with a mean MIC of 23.63±14.37% for all honeys tested. A fraction of samples tested (14.55%) resulted in an MIC of 6.25% whereas another fraction of samples tested (25.45%) resulted in an MIC of 12.50%. Manuka honey tested against *S. epidermidis* resulted in an MIC that ranged from 12.50-25.00%. Honey samples 4-(CITYMIX/WC), 16-(FYNBOS/WC), 18-(MIXEDGUM/FS), 19-(CITYMIX/FS), 20-(FORRESTREDGUM/WC), 51-(URBANFORAGE/WC), 52-(SUBURBANGARDEN/WC) and 160-(BOEKENHOUT/LIM) exhibited strong efficacy against *S. epidermidis* with all samples demonstrating an MIC value of 6.25%.

The data in this study also demonstrates that SA honey has antimicrobial activity against *S. aureus,* a pathogen commonly present in skin wounds (Tan et al., 2009). *S. aureus* has developed antibiotic resistance and is a clinical concern globally. MRSA is usually involved in difficult to treat skin and complicated underlying tissue infections (David and Daum, 2010). A study by Sherlock and co-workers (2010) underlined the testing of honey on *S. aureus* and reported that Ulmo honey (*Eucryphia cordifolia)* from Chile had MIC values of 3.1-6.3% ($^v/_v$) compared to the Manuka honey sample which had an MIC of 12.50% ($^v/_v$). French and co-workers (2005) reported that Manuka honey and pasture honey inhibited *S. aureus* at 2.70-5.00% ($^v/_v$). In a clinical investigation carried out by Simon and co-workers (2009), a 12 year old patient with cancer had a wound infected with MRSA and the wound was treated with a local antiseptic for 12 days. Despite this, the infection did not subside. The patient could not commence chemotherapy till the infection was cleared. After treatment with Medihoney®, a *leptospermum* honey, the infection subsided within two days (Tan et al., 2009). In general,

results from the majority of the samples tested in this study revealed that the laboratory reference strain (ATCC 25923) had a lower MIC inhibitory value than the clinical strain. A lack of substantial differences could be noted between the five different *Staphylococcus* strains tested, as mean MICs ranged between 25.23-27.73%. These *in vitro* results indicate that some honeys could potentially be utilised clinically against *S. aureus.* Honey samples 4-(CITYMIX/WC), 16–(FYNBOS/WC), 18–(MIXEDGUM/FS), 19–(CITYMIX/FS), 20–(FORRESTREDGUM/WC) and 52-(SUBURBANGARDEN/WC) demonstrated the greatest efficacy against all four *S. aureus* strains tested with all samples having an MIC value of 6.25% against all laboratory strains and clinical strains with the exception of 20–(FORRESTREDGUM/WC) and 52-(SUBURBANGARDEN/WC) which had an MIC of 12.50% against the clinical strains and 4-(CITYMIX/WC) had an MIC of 12.50% against the against the methicillin and gentamicin resistant *S. aureus.*

Candida spp. are considered to be one of the most significant opportunistic fungal pathogens and a common cause of skin disease (Vázquez et al., 2013). The majority of samples tested against *C. albicans* had an MIC value of 50.00%. The Manuka honey samples tested also had an MIC of 50.00% with the exception of one sample, 54-(MANUKA/NZ), which inhibited the laboratory strain at 25.00% and its clinical counterpart at 12.50%. Honey samples 19-(CITYMIX/FS) and 27-(AEF/WC) had an MIC of 6.25% and 12.5% respectively for the clinical strain of *C. albicans*. The antimicrobial efficacy of SA honeys tested are thus more sensitive to bacterial species than fungal species and this corresponds with previous studies (Basson and Grobler, 2008). Brady and co-workers (2004) conducted a survey of non-Manuka honey which revealed that the growth of *Candida* spp. was enhanced by selected honeys (Brady et al., 2004). This was possibly due to the presence of sugar which facilitated the fungal growth. A related study demonstrated that certain Iranian honeys required a 36.30% concentration or greater to be inhibitory against *C. albicans* (Khosravi et al., 2008). Hydrogen peroxide dependent honeys have been reported to be more effective against fungal organisms than non-peroxide honeys, such as Manuka honey (Irish et al., 2011).

2.3.2 Geographical location

Various studies on honey have shown that there are immense antimicrobial differences within different honeys and this is directly related to the different countries or regions from which they are sourced. The geographical location of the floral origin may affect the phytochemical composition and physiology of the floral species which essentially affects the honey which has been harvested from this flora. Diversity of the plant species is dependent on environmental factors such as sunlight, moisture and soil composition (Henriques et al.,

2005; Kaskoniene and Venskutonis, 2010). It has been postulated that the difference between countries can be attributed to varying compositions of pollen or nectar which has the greatest effect on honey composition (Kaskoniene and Venskutonis, 2010).

Karousou and co-workers (2005), demonstrated that essential oil composition in honey was dependent on geographical location even when compared within same plant species. Thus, the composition can be substantially different even for honeys of the same floral origin. This was further demonstrated by Irish and co-workers (2011), who reported a variation in hydrogen peroxide activity within the equivalent floral species confirming that environmental conditions in different regions play a role in the relationship between floral source and non-peroxide activity (Irish et al., 2011).

The honeys tested in this study were collected from various provinces in SA. Figure 2.5 demonstrates the geographical regions in SA where the honey samples were collect and the relative percentage of honey samples of the total collected from the respective regions.

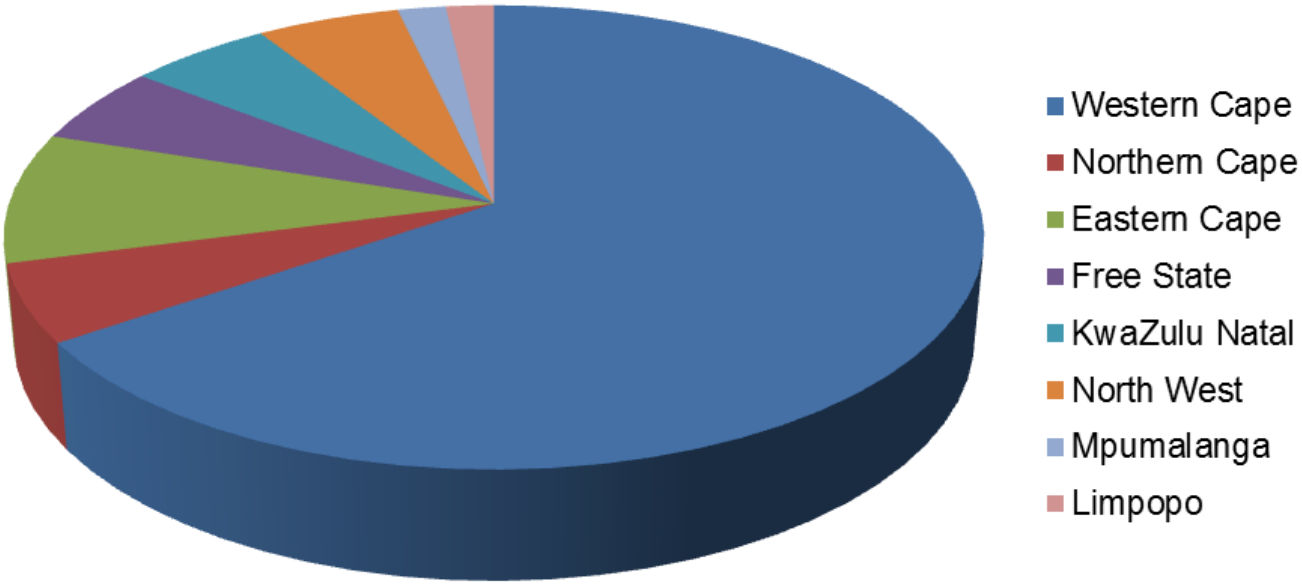


Figure 2.5: Graphical representation of the geographical regions in SA where the honey samples were collected and the relative percentage of honey samples of the total collection from the respective regions.

It was noted that the samples with the most favourable antimicrobial activity originate from the Western Cape {4-(CITYMIX/EC), 16-(FYNBOS/WC), 12-(BUSHVELD/KZN), 17-(AKMS/FS), 36-(EUCLADFICI/WC), 41-(INDIGENOUS/WC), 52-

(SUBURBANGARDEN/WC)} and the Free State {18-(MIXEDGUM/FS) and 19-(CITYMIX/FS)} in SA. This observation in turn may be due to the fact that the majority of the samples were collected from these regions.

Honeys with considerable antimicrobial activity may be obtained from a number of different environments. An interesting finding was that samples of the perceived equivalent floral species from the same province differed in antimicrobial activity. *Allium cepa* samples from the Western Cape confirmed this, whereby the sample from Outdshoorn (Sample 15-(ONION/WC) with a mean MIC of 15.28% displayed superior antimicrobial activity over sample 154-(ONION/WC), with a mean MIC of 50.00% obtained from Mossel Bay. Mossel Bay is at the coast and Oudtshoorn is 85 kilometres inland. The climate of the two cities differs, with higher temperatures reached in Oudtshoorn. The results thus demonstrate that geographical location plays a significant role in the honey's antimicrobial activity.

It has been further reported that honeys produced at one time in one location could vary in activity, suggesting that entomological factors play a substantial role (Irish et al., 2011). The health of individual bee colonies and the age of foraging workers could affect the foraging activity or the secretion of enzymes, thus affecting antimicrobial activity. Mandal and co-workers (2010) stated that the type of honey produced by the foragers is dependent on the natural vegetative flowers blooming in different seasons and places. Forager's preferences may result in the activity being affected due to it being produced from the nectar of numerous floral species. It is further documented by Ayaad and co-workers (2011) that the variation in the antimicrobial potency could be attributed to the source of the nectars which may have contributed to the difference in the antimicrobial honeys. Floral source determines many of the attributes of honey such as flavour, aroma, colour and composition.

Citrus Sample 33-(CITRUS/WC) from the Western Cape displayed similar antimicrobial properties in comparison to the Sample 49-(CITRUS/EC) from the Eastern Cape with the exception of investigations against *P. aeruginosa*. Sample 49-(CITRUS/EC) produced an MIC of 6.25% against *P. aeruginosa.* Fynbos samples (7-(FYNBOS/WC), 16-(FYNBOS/WC), 22-(FYNBOS/WC), 26-(FYNBOS/WC), 32-(FYNBOS/WC), 37-(FYNBOS/WC) and 45-(FYNBOS/WC) originate from different areas in the Western Cape, however, antimicrobial efficacy variation occurs, thus demonstrating that geographical location is a noteworthy variable. Sample 16-(FYNBOS/WC) with a mean MIC of 10.42±8.27, from Mossel Bay, demonstrated superior antimicrobial efficacy, followed by sample 45-(FYNBOS/WC) (mean MIC of 20.14±17.05) from Still Bay, 26-(FYNBOS/WC) (mean MIC of 20.14±17.05) from Cape Point, and 37-(FYNBOS/WC) (mean MIC of 26.39 ±14.5) from Stanford. However, other honey samples derived from the fynbos {Samples 7-

(FYNBOS/WC), 22-(FYNBOS/WC) and 32-((FYNBOS/WC)} displayed no noteworthy antimicrobial properties in comparison. Mossel Bay and Still Bay are closer to the coast, compared to Bainskloof, Somerset West, Herbertsdal and Stanford. The positioning of these areas result in varying climates thus affecting the chemical constituents of the honeys.

Eucalyptus cladocalyx samples 21-(SUGARGUM/WC) and 39-(SUGARGUM/WC) both originate from the Western Cape and both samples displayed similar antimicrobial efficacy. *Eucalyptus grandis* samples 47-(SALIGNAGUM/KZN) and 162-(SALIGNAGUM/KZN) both originate from Kwa-Zulu Natal displaying similar MIC values, however, no superior antimicrobial efficacy was noted. *Ziziphus mucronata* samples 13-(BUFFALOTHORN/NC) and 156-(BUFFALOTHORN/NC) originate from the Northern Cape and Samples 158-(BUFFALOTHORN/NW) and 159-(BUFFALOTHORN/NW) originate from the North West province. These four samples demonstrate equivalent inhibitory values with the exception of investigations against *P. aeruginosa* and the laboratory strain of *C. albicans* for honey sample 13-(BUFFALOTHORN/NC).

City mix Samples 4-(CITYMIX/WC) and 19-(CITYMIX/FS) which originated from the Western Cape and Free State provinces respectively had similar antimicrobial efficacy with the exception of studies against the clinical strain of *C. albicans* and the methicillin and gentamicin resistant *S. aureus* strain which was more sensitive to 19-(CITYMIX/FS) with an MIC of 6.25% contrasting to sample 4-(CITYMIX/WC) which had an MIC of 25.00% and 12.5% for the respective strains.

Strandveld samples 9-(STRANDVELD/WC), 11-(STRANDVELD/WC), 24-(STRANDVELD/WC), and 44-(STRANDVELD/WC) originate from different areas in the Western Cape. However, sample 44-(STRANDVELD/WC) from Albertina exhibited enhanced antimicrobial efficacy comparatively. Sample 9-(STRANDVELD/WC), 11-(STRANDVELD/WC), and 24-(STRANDVELD/WC) demonstrated no significant antimicrobial activity with the exception of 9-(STRANDVELD/WC), which resulted in an MIC of 6.25% against the *P. aeruginosa* strain.

Acacia mellifera sample 14-(HOOKTHORN/NC) from the Northern Cape and Sample 50-(HOOKTHORN/WC) from the Western Cape displayed equivalent inhibitory values of MICs of 50.00% for all pathogens tested.

2.3.3 Comparison of monofloral honeys to polyfloral honeys

The antimicrobial efficacy of honeys from a perceived monofloral source was comparatively evaluated with honeys perceived to have been obtained from a polyfloral source. City mix

samples {4-(CITYMIX/WC) and 19-(CITYMIX/FS)} both displayed superior antimicrobial efficacy with mean MIC values of 6.25% against S. *aureus*, MRSA*, P. aeruginosa* and *S. epidermidis*. This superior antimicrobial efficacy is diminished when city mix is combined with tuart gum 5-(CITYMIXTUART/WC) displaying a mean MIC value of 50.00%±00. *Allium cepa* 154-(ONION/WC) demonstrated a mean MIC value of 50.00% against all pathogens and sample 15-(ONION/WC) displayed an enhanced mean MIC of 15.28±5.51%. Despite this, the *Allium cepa* and *Erica* spp. sample {34-(ONION/FYNBOS)} demonstrated a mean MIC of 47.22%. Two *Erica* spp. {37-(FYNBOS/WC) and 40-(FYNBOSGUARRI/WC)} from Stanford were analysed. The monofloral sample 37-(FYNBOS/WC) exhibited better antimicrobial activity when compared to the polyfloral sample 40-(FYNBOSGUARRI/WC) which was a combination of *Erica* spp. and Euclea racemosa. A large variation in antimicrobial activity exists within monofloral *Erica* spp. samples. In combination with *Eucalyptus conferruminata* {sample 35-(FYNBOSEC/WC)} moderate antimicrobial activity was noted. Improved antimicrobial activity was noted in combination with *Morella cordifolia* (38-(WASBESSFYNBOS/WC). Two monofloral *Eucalyptus cladocalyx* samples {(21-(SUGARGUM/WC) and 39-(SUGARGUM/WC)} produced MIC values of 50.00 and 25.00%. Antimicrobial activity was noted in polyfloral sample 36-(EUCLADFICI/WC) whereby sugar gum was combined with *Eucalyptus ficifolia*. When *Eucalyptus cladocalyx* was combined with *Erica* spp. 8-(ECFYNBOS/WC) no marked improvement could be noted. Saligna gum 47-(SALIGNAGUM/KZN) and Karri gum 46-(KARRIGUM/WC) displayed an average MIC of 50% against all pathogens. Antimicrobial activity in mixed gums was substantially enhanced. Sample 18-(MIXEDGUM/FS) displayed an MIC of 6.25% of all strains with the exception of the *Candida* spp.

When examining the data from this study, the general trend seems to be that polyfloral honey has better activity than monofloral sources. This is not surprising as polyfloral sources could offer a far more complex range of chemical compounds, which might target the microbial organisms at multiple target sites and thus cause cell death or inhibition by differing mechanisms.

2.3.4 Honeys antimicrobial efficacy due to age

Many compounds in honey are unstable and their structures may transform during honey maturation and storage (Kaskoniene and Venskutonis, 2010). An example of the possibility of structural change was explored by Irish and co-workers (2011) who investigated the stability of honeys and revealed that non-peroxide honeys derived from clover, mixed flora and paperbark as well as samples from *L. liversidgei* either remained stable or declined with time. In addition, it was demonstrated that honey samples derived from another species; *L.*

polygalifolium, increased in antimicrobial activity. It is postulated that non-peroxide activity may increase in time as a resulting increase of methylglyoxal as an effect of the Maillard reaction (Irish et al, 2011). Allen and co-workers (1991) research conflicts, having reported that there is no correlation between the age of honey and its antimicrobial effects. The instability of the enzyme glucose oxidase may possibly have an effect on peroxide honeys. The stability and antimicrobial potency of honey over time has to be quantified to establish a shelf-life in honeys that have the potential to be commercialised. Honeys with phenomenal antimicrobial activity that degrade over time should not be excluded from being used clinically, as the efficacy of pharmaceutical products also diminishes with time. However, a suitable shelf-life could be established which would overcome this.

The MIC values of honey samples collected prior to 2011 are given in Table 2.5. In this study it was confirmed that *Erica* spp., *allium cepa* and *Eucalyptus tereticornis* older samples were less efficacious than newer samples. *Acacia mellifera, Eucalyptus cladocalyx, Eucalyptus grandis*, *Citrus* spp. displayed enhanced antimicrobial activity on ageing. Of the six Manuka honey samples analysed, two samples from 2008 and 2011 produced MIC values of 15.97 and 15.28 respectively. Two samples from 2008 and 2011 displayed mean MIC values of 20.14% and 20.17% respectively, thus proving that in this study on Manuka honey age was not a considerable factor affecting the antimicrobial efficacy of honey.

Table 2.5: Mean inhibitory concentration (% inhibition) of samples collected prior to 2011.

REFERENCE CODE	***S. aureus* ATCC 25923**	***S. aureus* clinical**	***S. aureus* (MRSA) 43300**	***S. aureus* (MRSA) clinical**	***S. aureus* (M & G)* ATCC 33592**	***S. epidermidis* ATCC2223**	***P. aeruginosa* ATCC9027**	***C. albicans* ATCC 10231**	***C. albicans* clinical**	**MEAN MIC**
60-(YELLOWBOX/WC)	50.00	50.00	25.00	25.00	50.00	50.00	50.00	50.00	50.00	44.44±11.02
61-(STRANDVELD/WC)	25.00	25.00	12.50	12.50	12.50	25.00	12.50	50.00	50.00	25.00±15.31
62-(SUGARGUM/WC)	12.50	12.50	12.50	12.50	12.50	6.25	6.25	50.00	50.00	20.31±18.53
64-(CITRUS/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
69-(OLIVEFYN/SC)	50.00	50.00	25.00	25.00	50.00	50.00	50.00	50.00	50.00	44.44±11.02
80-(FORESTREDGUM/WC)	25.00	25.00	25.00	25.00	6.25	25.00	6.25	50.00	50.00	26.39±15.56
86-(LITCHI/MP)	50.00	50.00	50.00	50.00	25.00	25.00	25.00	50.00	50.00	41.67±12.50

REFERENCE CODE	*S. aureus* ATCC 25923	*S. aureus* clinical	*S. aureus* (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC2223	*P. aeruginosa* ATCC9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
90-(FYNBOS/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
94-(HOOKTHORN/NC)	12.50	12.50	12.50	12.50	12.50	12.50	12.50	25.00	25.00	15.28±5.51
106-(ONION/WC)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
109-(FYNBOS/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
116-(FYNBOS/SC)	25.00	50.00	25.00	25.00	25.00	25.00	50.00	50.00	50.00	36.11±13.18
117-(WILDFLOWERS/GP)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
119-(BUFFALOTHORN/NW)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
123-(SALIGNAGUM/KZN)	25.00	25.00	25.00	25.000	12.50	12.50	12.50	50.00	50.00	26.39±14.58

REFERENCE CODE	*S. aureus* ATCC 25923	*S. aureus* clinical	*S. aureus* (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC2223	*P. aeruginosa* ATCC9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
135-(FYNBOS/WC)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	50.00	50.00	30.56±11.02
139-(BLUEBUSH/NW)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
142-BUFFALOTHORN/NW)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
144-(FYNBOS/WC)	12.50	50.00	12.50	12.50	12.50	12.50	12.50	50.00	50.00	25.00±18.75
145-(COSMOS/NP)	25.00	50.00	50.00	50.00	25.00	6.25	12.50	50.00	50.00	35.42±18.22
146-(REDSYRING/LIM)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
148-(ALOE/NP)	50.00	50.00	50.00	25.00	12.50	6.25	12.50	50.00	50.00	34.03±19.54
149-(HOOKTHORN/NP)	50.00	50.00	50.00	50.00	50.00	25.00	50.00	50.00	50.00	47.22±8.33

REFERENCE CODE	*S. aureus* ATCC 25923	*S. aureus* clinical	*S. aureus* (MRSA) 43300	*S. aureus* (MRSA) clinical	*S. aureus* (M & G)* ATCC 33592	*S. epidermidis* ATCC2223	*P. aeruginosa* ATCC9027	*C. albicans* ATCC 10231	*C. albicans* clinical	MEAN MIC
150-(COMBRETUM/GP)	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00±0.00
155-(CATTHORN/EC)	50.00	50.00	50.00	50.00	50.00	50.00	6.25	50.00	50.00	45.14±14.58
96-(MANUKA/NZ)	6.25	6.25	6.25	6.25	6.25	25.00	6.25	50.00	50.00	15.97± 19.29
97-(MANUKA/NZ)	6.25	6.25	6.25	6.25	6.25	25.00	25.00	50.00	50.00	20.14±18.69
147-(MANUKA/NZ)	50.00	50.00	25.00	25.00	12.50	12.50	12.50	50.00	50.00	31.94± 17.8

2.4 Conclusions

- The antimicrobial activity of 55 honey samples collected in 2011 demonstrated % inhibition ranging from 10.42-50.00%.
- The control, Manuka honeys, displayed % inhibition ranging from 15.28-41.67%.
- The greatest antimicrobial activity was exhibited by honey sample 16-(FYNBOS/WC) which displayed a mean MIC of 10.42±8.27%.
- Nine honey samples displayed a mean MIC value that was superior to that of the lowest mean MIC value exhibited by the control, Manuka honey.
- Polyfloral honey displayed better activity than monofloral sources.
- An enhanced antimicrobial activity was displayed on ageing, in certain samples.
- Results of this study confirm that SA honeys do possess antimicrobial efficacy.

CHAPTER 3
ANTIBIOTIC-HONEY COMBINATION STUDIES

3.1 Introduction

Infectious diseases are a multifaceted interplay between the pathogen, the physiological and immunological status of the host and the environment (Guillemot, 1999). The increased, improper and irrational utilisation of antibiotics by prescribers has precipitated towards world-wide antibiotic resistance. Prescribers using treatment regimens based on empiric therapy as opposed to definitive therapy has greatly contributed to antibiotic resistance. Furthermore, patients also contribute to antibiotic resistance by improper usage, such as missing doses and not completing the entire course of antibiotics as prescribed. In the current era of vaccines and antibiotics, one would expect that infectious diseases be under control, however, in many developing countries they continue to be principle causes of mortality at an alarming rate (WHO, 1999).

This has financial consequences as there may be a need for further laboratory testing, utilisation of more drugs, longer treatment duration, and increased hospitalisation (Nyasulu et al., 2012). Conventional treatment options may be more costly and ease of access to these options may prove arduous. Apart from resistance being a major concern, antibiotics themselves, demonstrate adverse effects such as hypersensitivity or allergic reactions, nausea, ototoxicity, immunosuppression and destruction of good intestinal flora (Al-Jabri, 2005). This has resulted in research efforts to determine if complementary and alternative medication (CAM) such as honey, could be an effective antimicrobial agent when incorporated or utilised with conventional antimicrobials as a pharmaceutical healthcare option. These preparations should display low toxicity and have a little to no environmental impact (Darwish and Aburjai, 2010).

Infections with antibiotic resistant pathogens are a major healthcare concern. Despite this, there are limited new antibiotics in development, as seen in the decreased numbers of regulatory approvals. The lack of profitability and the challenge of finding novel targets for antibiotics in pathogens, contribute to the deficiency of new antibiotics in development. Resistance generally occurs via target modification, efflux, immunity and bypass, and enzyme catalysed destruction (Wright, 2010). The persistent use of antibiotics results in mutation of the pathogens culminating in possible resistance to the antibiotic.

Gallocatechins, *Salivia milttiorhiza*, curcuminoids, carnosic acid, berberine, aqueous crude khat extracts, and diterpenes from *Lycopus europaues* are only some of the natural products currently being investigated for possible combination therapy with antibiotics (Hemaiswarya et al., 2008; Jenkins and Cooper, 2012). CAM agents could also be economically advantageous, may possess superior patient acceptability and are not stringently regulated. In practice, patients often utilise CAM with conventional medication concurrently. It is postulated that honey possesses chemical components similar to that of antibiotics (Brudzynski and Lannigan, 2012). Natural products for medicinal use present an innumerable variety of chemical structures which may serve as lead molecules whose activities can be further improved through combinations with chemicals and by synthetic chemistry. Several natural products are selected for their antimicrobial effects and are utilised in combination with conventional antibiotics. There are numerous advantages of utilising natural products in combination with antibiotics. Natural products have different mechanisms of action which could either enhance the antibiotics mechanism of action or provide an additional mechanism, resulting in dose reduction, quicker onset of action and a decreased duration of therapy. This could significantly decrease adverse side effects that are usually associated with high doses of antibiotics, ultimately delaying or preventing antimicrobial resistance (Hemaiswarya et al., 2008). For example when honey and bovine milk are used in combination there was a quicker onset of action in comparison to the individual components (Al-Jabri, 2005). SA honey may potentially be synergistic, additive or indifferent with conventional antibiotics, as certain SA honeys demonstrate noteworthy antimicrobial potency as demonstrated in Chapter 2 of this research report.

In this Chapter, combination studies were undertaken whereby the conventional antimicrobials; ciprofloxacin, gentamicin and antifungal agents; amphotericin B and nystatin were combined with a selection of SA honeys to investigate whether synergism, additive or antagonistic interactions were evident.

3.2 Materials and methods

3.2.1 Honey samples

To evaluate the possible synergistic effects of SA honey with conventional antibiotics, five honey samples were selected based on their enhanced antimicrobial efficacy as observed in Chapter 2. Honey samples utilised in this study included; 16-(FYNBOS/WC), 18-(MIXEDGUM/FS), 19-(CITYMIX/FS), 26-(FYNBOS/WC) and 41-(INDIGENOUS/WC).

3.2.2 Pathogens and antibiotics

Ciprofloxacin and gentamicin were selected as test antibiotics for this study. Similarly, two antifungals (nystatin and amphotericin B) were also selected. Antibiotics and antifungals were purchased from Sigma Aldrich. Ciprofloxacin and gentamicin were tested against *Pseudomonas aeruginosa* (laboratory – ATCC 9027) and *Staphylococcus aureus* (laboratory - ATCC 25923). Nystatin and amphotericin B were tested against *Candida albicans* (laboratory – ATCC 10231). Stock solutions of antibiotics were appropriately prepared with an antibiotic starting concentration of 0.01mg/mL and an antifungal starting concentration of 0.001 mg/mL. These antimicrobial agents are classified as broad-spectrum agents and were selected because they offer greater pathogen coverage. This will subsequently elucidate the synergistic, additive or antagonistic effects of combination with honey over a wider range of pathogens and thus offer a more holistic account of these effects.

3.2.3 MIC determination

The microtitre plate microdilution method was used to investigate antimicrobial activity of honey combined with conventional antimicrobials (ciprofloxacin, gentamicin for bacteria and nystatin and amphotericin B for yeasts). Methods were adapted from National Committee for Clinical Laboratory Services guidelines (NCCLS, 2003) with modifications catering for the viscosity of honey. Figure 3.1 is a typical 96-well microtitre plate that was adapted for this study. This method is advantageous as it is timeous, economical and the results are reproducible. When using Figure 3.1 as a template, under aseptic conditions (laminar flow), all wells marked from row B-H of the 96-well microtitre plate (NUNC, Denmark) were filled with 100 µL of sterilized deionised water. Wells A1 – A3 were filled with 200 µl of 50% honey. Wells A4-A6 were filled with 100 µl of antibiotic and 100 µl of sterile deionised water. Wells A7-A9 were filled with 100 µl of antibiotic and 100 µl of 50% honey solution. Serial doubling dilutions were then performed. The microtitre plates were then removed from the laminar flow unit. A 100 µl of TSB inoculated with the respective pathogen (1:100) was subsequently added to each well. A Gram-negative reference strain (*P. aeruginosa* NCTC 9027), Gram-positive reference strain (*S. aureus* ATCC 25923) and yeast (*C. albicans* ATCC 10231) were selected to test the antimicrobial efficacy of honey samples in combination with conventional antibiotics and antifungals. The microtitre plates inoculated with bacterial pathogens were incubated at 37 °C for 24 hours whereas the microtitre plates inoculated with the yeast were incubated for 48 hours at 37 °C. Negative controls were attained by utilising a honey/drug free well (A10-A12).

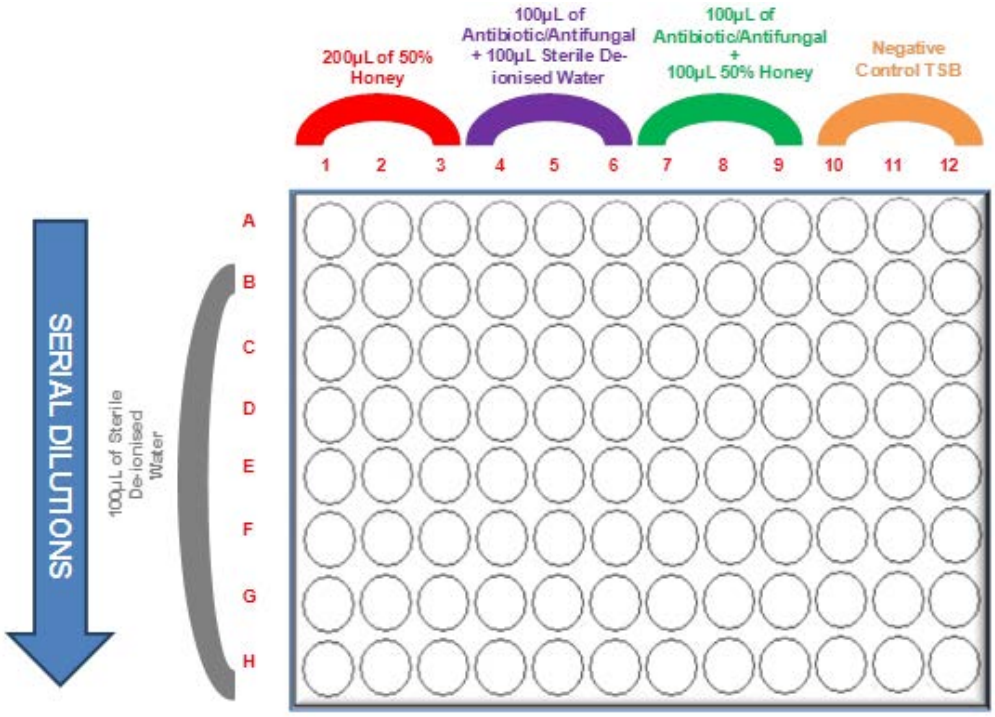


Figure 3.1: Serial dilutions utilising a 96-well microplate adapted from NCCLS guidelines (2003) with modifications catering for the viscosity of honey.

A streak plate of the culture was also incubated, to ensure purity of the culture by identification of single colonies. To determine if the media utilised was sterile, the media was left overnight at room temperature. A visual inspection for contamination i.e. turbidity, was used to confirm the sterility of the media.

After appropriate incubation periods, 40 µl of iodonitrotetrazolium violet (Sigma-Aldrich) (INT) indicator was added to each well to improve visual identification of microbial growth or inhibition of test pathogens. INT indicator solution was prepared by weighing 0.04 g of INT and adding this to 100 mL of sterile distilled water. Due to the fact that INT is insoluble in cold water, it was placed in a shaker incubator at 55 °C for 30 minutes. It was then stored in the refrigerator at 4 °C. The colourless tetrazolium salt is reduced to a red colour by acting as an electron acceptor by metabolically active micro-organisms (Eloff, 1998). Where there was no microbial growth, INT remained colourless.

The MIC was defined as the lowest concentration of honey, antibiotic or honey-antibiotic combination to inhibit visible growth. A light box was utilised to facilitate recognition of colour differences.

3.2.4 Determination of Fractional Inhibitory Concentration (FIC) Index

The i*n vitro* antimicrobial interaction was assessed utilising the FIC index (ΣFIC). The Loewe additivity zero-interaction theory forms the basis for FIC determination. It is hypothesised that because a drug cannot interact with itself, the effect of self-drug combination will always be additive depicting a FIC value of 1. This is a prelude to the hypothesis that less or more drug would be required in order to produce the same effects as the drug alone (Meletiadis et al., 2010). The FIC value was used to establish if honey and antibiotics are synergistic, additive, indifferent or antagonistic when combined together and were calculated using Equation 1 and 2 which were adapted from Van Vuuren and Viljoen (2011). The ΣFIC is a summation of the FIC of the individual components being analysed. The FIC of each component was determined by dividing the MIC of the agent when used in combination over the MIC of the agent when utilised alone (Equations 1 and 2). The ΣFIC was used to assess interactive antimicrobial effects for honey and antibiotic combinations.

$$\mathrm{FIC(i)} = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) independently}} \qquad \textit{Equation 1}$$

$$\mathrm{FIC(ii)} = \frac{\text{MIC (b) in combination with (a)}}{\text{MIC (b) independently}} \qquad \textit{Equation 2}$$

Where, (a) represents the honey, (b) the respective antimicrobial and (i) and (ii) represents the honey and the antimicrobial in combination

The sum of the FIC, known as the FIC index is thus calculated as:

$$\Sigma\mathrm{FIC} = \mathrm{FIC}(*i) + \mathrm{FIC}(*ii) \qquad \textit{Equation 3}$$

The FIC index was used to determine the correlation between the honey and conventional agent. An FIC index ≤0.5 is indicative of synergism, whereas a FIC index > 4.0 is an indication of antagonism. Additive effects are exhibited when the FIC index ranges from > 0.5-1.0. Indifferent effects are depicted when the calculated FIC is >1.0 and ≤4.0 (Van Vuuren and Viljoen, 2011). Synergism would occur when honey and antibiotics used in combination have enhanced effects in comparison to being used individually, greater than additive effects. If the combined effects result in deterioration of activity, the combination would then be classified as antagonistic.

3.3 Results and discussion

Table 3.2-3.5 describes honey-antibiotic combinations tested against each pathogen listing the MIC of the antibiotic, honey and the honey-antibiotic combination, FIC value and interpretation of the FIC value.

3.3.1 Combination with ciprofloxacin

S. aureus is one of the most common wound pathogens frequently isolated in wounds and is the pathogen responsible for a multitude of acute and chronic skin infections (Muller et al., 2013). Resistance of ciprofloxacin to *S. aureus* especially MRSA has increased to over 90% (Limoncu et al., 2003). Although a lack of synergy was demonstrated with ciprofloxacin in combination with honey, additive effects were seen. These additive effects were demonstrated when honey samples 18-(MIXEDGUM/FS), 19-(CITYMIX/FS), 41-(INDIGENOUS/WC) were combined with ciprofloxacin and tested against *S. aureus*. Similarly, honey samples 16-(FYNBOS/WC), 26-(FYNBOS/WC) and 41-(INDIGENOUS/WC) displayed additive effects with ciprofloxacin against *P. aeruginosa*. Additive interactions may prove to be valuable in the prevention or delay of further resistance.

Reports of resistance to ciprofloxacin have been documented with mechanisms of resistance to ciprofloxacin generally as a result of mutations of topoisomerase II, topoisomerase IV and/ or activation of drug efflux pump (Limoncu et al., 2003). Ranjbar and co-workers (2011) reported an incidence of 65% resistance of ciprofloxacin to 70 isolated strains of *P. aeruginosa* in infected burn wounds (Ranjbar et al, 2011). Valencia and co-workers reported increases in quinolone resistant *P. aeruginosa* in infected leg ulcers (Valencia et al., 2004). In this study, two synergistic combinations were demonstrated for honey samples 18-(MIXEDGUM/FS) and 19-(CITYMIX/FS) in combination with ciprofloxacin against *P. aeruginosa* (Table 3.1). The samples displayed a ΣFIC of 0.38 and 0.50 respectively confirming their synergistic action. Ciprofloxacin (µg/mL): Honey (%) ratios utilised in these synergistic combinations were 0.00375:0.0375 for 18-(MIXEDGUM/FS) and 0.005: 0.05 for 19-(CITYMIX/FS). The results corroborates with a previous study whereby enhanced activity of ciprofloxacin was also seen in combination with honey against *P. aeruginosa* (Abd-el et al., 2007).

Table 3.1: MIC and ΣFIC values of honey samples with ciprofloxacin.

PATHOGEN	HONEY SAMPLE	MIC			FIC	
		CIPROFLOXACIN µG/ML	HONEY %	COMBINATION CIPROFLOXACIN (µG/ML): HONEY (%)	ΣFIC	INTERPRETATION
S. aureus (laboratory - ATCC 25923)	16-(FYNBOS/WC)	0.313	25.00	0.313 3.13	1.13	Indifferent
	18-(MIXEDGUM/FS)	0.313	25.00	0.156 1.56	0.56	Additive
	19-(CITYMIX/FS)	0.313	25.00	0.156 1.56	0.56	Additive
	26-(FYNBOS/WC)	0.313	25.00	0.313 3.13	1.13	Indifferent
	41-(INDIGENOUS/WC)	0.313	25.00	0.156 1.56	0.56	Additive
P. aeruginosa (laboratory – ATCC 9027)	16-(FYNBOS/WC)	0.008	12.50	0.005 0.05	0.67	Additive
	18-(MIXEDGUM/FS0	0.010	25.00	0.00375 0.0375	**0.38**	**Synergistic**
	19-(CITYMIX/FS)	0.010	25.00	0.005 0.05	**0.50**	**Synergistic**
	26-(FYNBOS/WC)	0.008	25.00	0.010 0.100	1.33	Additive
	41-(INDIGENOUS/WC)	0.010	25.00	0.010 0.100	1.00	Additive

3.3.2 Combination with gentamicin

In this study, synergistic effects with gentamicin against *S. aureus* were observed with honey samples; 16-(FYNBOS/WC), 18-(MIXEDGUM/FS), 19-(CITYMIX/FS), 26-(FYNBOS/WC) and 41-(INDIGENOUS/WC) (Table 3.2). Synergism of gentamicin with honey samples 16-(FYNBOS/WC) and 19-(CITYMIX/FS) was also observed against *P. aeruginosa*. The synergy demonstrated by all five honey samples in combination with gentamicin against *S. aureus* is noteworthy. Furthermore, additive effects of gentamicin with honey samples 18-(MIXEDGUM/FS), 26-(FYNBOS/WC) and 41-(INDIGENOUS/WC) against *P. aeruginosa* were demonstrated. As previously mentioned, although not as desirable as synergy, additive effects could curb resistance mechanisms.

P. aeruginosa is an important pathogen which is usually acquired nosocomially and is a serious cause of infection (Abd-el et al., 2007). This pathogen is of clinical significance as it can result in death, particularly in the immunocompromised and burn patients. It is also known to be resistant to antimicrobial therapy (Karakoc and Gerceker, 2001, Mullai and Menon, 2005). A number of *P. aeruginosa* strains have been reported to show resistance to several antibiotics and serious *P. aeruginosa* infections are now generally being treated with antibiotic multitherapy (Abd-El et al., 2007; Jayaram et al., 2010). These antibiotics usually have different targets to enhance antibacterial activity. Two of the five honeys namely; 16-(FYNBOS/WC) and 19-(CITYMIX/FS) demonstrated synergy in combination with gentamicin. In a previous study, Karayil and co-workers (1998) successfully reported the synergistic effect of Indian honey with gentamicin, amikacin and ceftazidime against six multi-drug resistant *Pseudomonas* strains utilising the broth dilution method. Synergism was not produced against the eight *Klebsiella* strains tested (Karayil et al., 1998).

Al-Jabri (2005) reported that honey and gentamicin in combination, achieved a better bactericidal effect than when compared to the antibiotic and the honey tested individually (Al-Jabri, 2005). The study tested 30 Omani honeys, of which one sample demonstrated an enhanced activity of gentamicin against *S. aureus* by 22% in 30 minutes (Al-Jabri, 2005). Synergism between gentamicin and honey was not reproduced in a study performed by Jenkins and Cooper (2012). This was possibly due to the fact that different honeys from different geographical regions were studied (Jenkins and Cooper, 2012).

Table 3.2: MIC and ΣFIC values of honey samples with gentamicin.

PATHOGEN	HONEY SAMPLE	MIC				FIC	
		GENTAMICIN µG/ML	HONEY %	COMBINATION GENTAMICIN (µG/ML): HONEY (%)		ΣFIC	INTERPRETATION
S. aureus (laboratory - ATCC 25923)	16-(FYNBOS/WC)	0.156	25.00	0.039	0.39	**0.27**	**Synergistic**
	18-(MIXEDGUM/FS)	0.156	25.00	0.039	0.39	**0.27**	**Synergistic**
	19-(CITYMIX/FS)	0.156	25.00	0.039	0.39	**0.27**	**Synergistic**
	26-(FYNBOS/WC)	0.156	25.00	0.039	0.39	**0.27**	**Synergistic**
	41-(INDIGENOUS/WC)	0.156	25.00	0.039	0.39	**0.27**	**Synergistic**
P. aeruginosa (laboratory – ATCC 9027)	16-(FYNBOS/WC)	0.625	25.00	0.156	1.56	**0.31**	**Synergistic**
	18-(MIXEDGUM/FS)	0.625	25.00	0.313	3.13	0.63	Additive
	19-(CITYMIX/FS)	0.625	25.00	0.235	2.35	**0.47**	**Synergistic**
	26-(FYNBOS/WC)	0.625	25.00	0.313	3.13	0.63	Additive
	41-(INDIGENOUS/WC)	0.625	25.00	0.313	3.13	0.63	Additive

3.3.3 Combination with nystatin

Synergism of nystatin with the 16-(FYNBOS/WC) honey sample against *C. albicans* was noted whereas additive effects of nystatin with honey samples were observed with combinations containing 18-(MIXEDGUM/FS), 19-(CITYMIX/FS), 26-(FYNBOS/WC) and 41-(INDIGENOUS/WC) honeys. Nystatin is a polyene antifungal agent which acts by binding with ergosterol in fungal membranes resulting in changes to the permeability of the membrane consequently leading to intracellular leakage of components causing cell death (Ashley et al., 2006).

Candida infections are on the rise as a result of the utilisation of broad-spectrum antibiotics and the increased number of patients that are immunocompromised as a result of the HIV epidemic (Álvaro-Meca et al., 2013). Owing to this fact, a CAM with possible antifungal effects such as honey would curb resistance when used in combination with a conventional honey.

Table 3.3: MIC and FIC values of honey samples with nystatin.

PATHOGEN	HONEY SAMPLE	MIC				FIC	
		NYSTATIN µG/ML	HONEY %	COMBINATION NYSTATIN (µG/ML): HONEY (%)		ΣFIC	INTERPRETATION
C. albicans (laboratory – ATCC 10231)	16-(FYNBOS/WC)	0.00625	12.50	0.00156	1.56	**0.37**	**Synergistic**
	18-(MIXEDGUM/FS)	0.009375	25.00	0.00625	6.25	0.92	Additive
	19-(CITYMIX/FS)	0.00625	25.00	0.00625	6.25	1.25	Additive
	26-(FYNBOS/WC)	0.00625	25.00	0.00312	3.13	0.62	Additive
	41-(INDIGENOUS/WC)	0.00625	25.00	0.00625	6.25	1.25	Additive

3.3.4 Combination with amphotericin B

In this study additive effects of amphotericin B with honey samples 16-(FYNBOS/WC); 18-(MIXEDGUM/FS), 19-(CITYMIX/FS), 26-(FYNBOS/WC) and 41-(INDIGENOUS/WC) were observed (Table 3.4).

Amphotericin B exerts its activity similarly to nystatin. This is achieved by forming complexes with sterols, mainly ergostreol, increasing permeability, resulting in intracellular leakage of components and cell mortality. Amphotericin B is a broad-spectrum antifungal agent and is generally the drug selected for treating systemic mycoses (Ashley et al., 2006, SAMF, 2012) Its indications include; disseminated candiasis, cryptococcis, mucormycosis, histoplasmosis, extracutaneous sporotrichhosis, blastomycosis and leishmaniasis (SAMF, 2012).

Antifungal agents are often associated with toxicities. Additive interactions with honey may facilitate a dose reduction in the antifungal utilised, thereby aiding to decrease adverse effects and toxicities associated with antifungal agents. A major concern is the economic impact of treating with newer antifungal agents due to their costly nature (Munoz et al., 2010). Honey may provide an economical advantage as it is by far, a cheaper option. Additive interactions maybe helpful to reduce the financial burden associated with fungal infections.

Table 3.4: MIC and FIC values of honey samples with amphotericin B.

PATHOGEN	HONEY SAMPLE	MIC				FIC	
		AMPHOTERICIN B µG/ML	HONEY %	COMBINATION NYSTATIN (µG/ML): HONEY (%)		ΣFIC	INTERPRETATION
C. albicans (laboratory – ATCC 10231)	16-(FYNBOS/WC)	0.0002	1.56	0.0002	0.20	1.13	Additive
	18-(MIXEDGUM/FS)	0.0002	25.00	0.0002	0.20	1.00	Additive
	19-(CITYMIX/FS)	0.0002	25.00	0.0002	0.20	1.00	Additive
	26-(FYNBOS/WC)	0.00039	25.00	0.00039	0.39	1.02	Additive
	41-(INDIGENOUS/WC)	0.00039	25.00	0.00039	0.39	1.02	Additive

3.3.5 Overview

Honey is a beneficial natural product to be investigated as it has been identified to be a complex substance comprising numerous components with antibacterial properties such as methylglyoxal and hydrogen peroxide. Antibacterial properties of honey can be attributed to various aspects, producing a bactericidal effect (Mathews and Binnington, 2002). Physicochemical properties such as pH, moisture content and sugar content further contribute to the antibacterial properties. Honey also promotes wound healing and can act as a highly viscous physical barrier protecting the wound surface from becoming colonised and invaded by potential pathogens.

This research investigated thirty combinations of honey and antibiotic, which revealed ten synergistic combinations, eighteen additive combinations, two indifferent combinations and most importantly, no antagonistic combinations. The most effective synergistic combinations were demonstrated by all five honeys tested in combination with the antibiotic gentamicin against *S. aureus*. Synergy was also observed in ciprofloxacin combined with 18-(MIXEDGUM/FS) and 19-(CITYMIX/FS) against *P. aeruginosa;* gentamicin combined with 16-(FYNBOS/WC) and 19-(CITYMIX/FS) against *P. aeruginosa;* nystatin combined with 16-(FYNBOS/WC) against *C. albicans.* No antagonistic interactions were demonstrated.

Synergistic combinations show a decrease in the MIC value of the antibiotic when combined with honey. This would allow for dose reduction of the antibiotic thereby minimising possible side effects, reducing treatment costs and providing a therapeutic option with greater antimicrobial potential. In addition, the potential risk of antimicrobial resistance is thought to be minimised with the utilisation of combination therapy.

Other studies reporting synergistic interactions of honey and antibiotics include the study by Jenkins and Cooper (2012) where Manuka honey combined with imipenem, tetracycline and mupirocin were tested against MRSA (Jenkins and Cooper, 2012).

Muller and co-workers investigated the combination of Medihoney® and rifampicin. Rifampicin is utilised in the treatment of TB, leprosy, brucellosis and resistant *Staphylococcal* infections (SAMF, 2012). The study revealed that the honey-rifampicin combination was synergistic against MRSA and clinical isolates (Muller et al., 2013). A key observation was that if MRSA was treated with rifampicin only, the pathogen rendered resistance hastily. However, the *in vitro* combination treatment did not result in rifampicin-resistant MRSA (Muller et al., 2013). This could be due to the complex nature of honey and its numerous components offering varying modes of action.

Methylglyoxal is postulated to be the main component contributing to antibacterial activity, however research has indicated that methylglyoxal individually does not produce a synergistic effect in combination with rifampicin while Medihoney® in combination with rifampicin produced a synergistic effect (Muller et al., 2013). This confirms that the numerous components of honey together produce an antibacterial effect.

Although this was entirely an *in vitro* study, the ramifications cannot be underestimated. There is potential to develop a therapeutically effective combination therapy to improve bacteriostatic and bactericidal mechanisms to rapidly eradicate infections. In addition, it is hypothesised that this approach could prevent or delay the global concerns of antimicrobial resistance. Infections with antibiotic resistant pathogens progress rapidly and are becoming increasingly difficult to treat. These infections are associated with morbidity and mortality. Despite this, there are limited novel antibiotics on the horizon. Resistance is more easily developed with therapeutic utilisation of a single agent in comparison to multiple agents, hence, a combination of agents is required. Insurmountable benefits arise from combining a conventional antimicrobial with a complementary antimicrobial agent. Results from this study emphasise the potential of combining honey with a conventional antibiotic as no antagonistic or deleterious effects were demonstrated.

3.4 Conclusions

- Synergism was demonstrated between honeys; 18-(MIXEDGUM/FS) and 19-(CITYMIX/FS) with the broad-spectrum antibiotic ciprofloxacin against *P. aeruginosa.*

- Synergistic effects with gentamicin against *S. aureus* were observed with honey samples; 16-(FYNBOS/WC), 18-(MIXEDGUM/FS), 19-(CITYMIX/FS), 26-(FYNBOS/WC) and 41-(INDIGENOUS/WC).
- Two of the five honeys namely; 16-(FYNBOS/WC) and 19-(CITYMIX/FS) demonstrated synergy in combination with gentamicin
- Synergistic effects between the honey sample 16-(FYNBOS/WC) and the antifungal nystatin were elucidated against *C. albicans.*
- The combination of Amphotericin B with the selected SA honeys yielded only additive effects.
- No antagonistic interactions were observed for all combinations tested.

CHAPTER 4
PHYSICOCHEMICAL PROPERTIES

4.1 Introduction

Honey comprises glucose and fructose as majority components and a much lower water component thus resulting in a notable interaction between these sugars and the water molecules. In turn, this results in fewer free water molecules available for microbial growth (Olaitan et al., 2007). These properties additionally contribute to honeys wound healing characteristics. The moist environment provided by honey promotes wound healing by stimulating tissue regrowth and anti-inflammatory effects are also observed (Molan (a), 1999). The viscous nature of honey provides a protective barrier to prevent infection and cross contamination by sealing the wound (Molan, 2002). Fibroblast activity in terms of migration, proliferation, and organisation of collagen requires a mildly acidic wound environment (Lusby et al., 2002). This activity may be optimised and maintained by the pH of honey which aids in achieving this type of environment.

The chemical constituents contribute to the physicohemical properties of honey. These properties impart on to honey its characteristic quality and antimicrobial activities (Manyi-Loh et al., 2012). The market demand for selected honey of high therapeutic value has increased, creating the need for honey to be standardised and authenticated (Brady et al., 2004). Globalisation of the honey market also contributes to the need of a standardised honey.

The sugars present in honey are as a result of enzymatic action on nectar sucrose (Ouchemoukh et al., 2010). The primary sugars contained in honey are glucose and fructose. Glucose and fructose are monosaccharides, consisting of a single carbon backbone (Olaitan et al., 2007). Honey also contains disaccharides such as maltose, sucrose, isomaltose. Furthermore, oligosaccharides are also componential elements.

The water content of honey is also an important variable, which is affected by numerous factors. The moist environment offered by honey protects the wound, reduces infection rates, debrides necrotic tissue, promotes granulation tissue formation and is an effective analgesic (Lusby et al., 2005).

Adulteration of honey with low cost sugar syrup or artificial honey occurs frequently (Wang and Li; 2011). Mislabelling of honeys from different geographical areas also poses a

problem. Many natural products that are expensive and produced under wide harvesting conditions and varying weather patterns are the most prone to adulteration (Mehryar and Esmaiili, 2011). Isotope analysis of honey has thus gained increasing popularity to authenticate honey and is being recognised as an established method for identifying honey adulteration (Chesson et al., 2010). The above-mentioned examples are examples of deliberate altering of honey for economic benefits. Despite this, adulteration may also occur naturally or due to human error. To cater for the sensitivity of the matter, the word "impurification" was utilised in this study. Impurification is a generic term encompassing changes to honeys properties either occurring due to natural phenomenon, by human error or the more sensitive, deliberate alteration for financial gain (adulteration).

The principle aim of this chapter was to gain knowledge and insight into the chemical composition and physical parameters of SA honeys. The accompanied physicochemical data acquired in this study is of great importance, as only two studies have investigated the physicochemical properties of SA honey (Manyi-loh et al., 2012; Serem and Bester, 2012). There is limited data on the physicochemical properties of honey, despite its use in the food industry, health products and production of by-products such as wax and propolis. Analysing different isotope profiles of SA honeys may provide a "finger print" for each honey sample as well as confirmation that the samples are not adulterated or impurified.

4.2 Materials and methods

4.2.1 Honey samples

The physicochemical testing was conducted on 36 honey samples of various botanical origins which were collected in 2011 based on their availability. None of the honey samples showed signs of fermentation or crystallisation prior to analysis of physicochemical properties demonstrating that these samples maintained their integrity and were devoid of deterioration. Samples were selected on the basis of antimicrobial efficacy (previous chapters) and availability.

4.2.2 Measurement of physicochemical properties

The water content and sugar content of whole honey was measured with a honey refractometer. The pH was determined using a pH meter by dissolving 10 g of whole honey in 75 mL of distilled water, following standard AOAC 998.12 protocols (AOAC 998.12, 1999). Impurifications of honeys were also quantified. Apparent $\%C_4$ impurification, where values

greater than 7% were considered questionable was calculated for each honey (White and Winters, 1989) utilising Equation 4:

$$\text{Impurification (\%)} = \frac{[(\delta^{13}C \text{ (protein)} - (\delta^{13}C \text{ (whole honey)}]*100 /}{[(\delta^{13}C \text{ (protein)} - (-9.7)]}$$

Equation 4

4.3 Results and discussion

Table 4.1 presents the results obtained for the physicochemical parameters (pH, sugar content, moisture content) of the commercially purchased Manuka honey samples tested (controls) and Table 4.2 summarises the results of selected SA honeys. The mean MICs are included in Table 4.1 and Table 4.2 for reference purposes as the antimicrobial activity of honey is known to be a result of its high sugar content, acidic nature and low moisture content (Lusby et al., 2005; Mandal et al., 2010). The Manuka honey samples had a water content ranging from 17.40-19.00% and a pH range of 4.28-4.44. In addition, the samples also displayed a sugar range of 79.25-81.00% and a percentage impurification ranging from 0.38-14.51 (Table 4.1). Surprisingly, the purchased Manuka honey sample 56-(MANUKA/NZ) demonstrated substantial impurification. No considerable differences were noted between the SA samples and the Manuka honey.

Table 4.1: Physicochemical properties and mean MIC of Manuka samples tested.

REFERENCE CODE	WATER CONTENT %	PH	SUGAR CONTENT %	% IMPURIFIED/ADULTERED	MEAN MIC (%)
54-(MANUKA/NZ)	18.50	4.44	79.50	0.95	15.28
55-(MANUKA/NZ)	17.40	4.28	81.00	0.38	20.17
56-(MANUKA/NZ)	19.00	NES	79.25	14.51	41.67
MEAN	18.30 ± 0.82	4.36 ± 0.11	79.92 ± 0.95	5.29 ± 8.01	

NES-Not Enough Sample for analysis

Table 4.2: Physicochemical properties and mean MIC of selected honey samples.

REFERENCE CODE	WATER CONTENT %	PH	SUGAR CONTENT %	% IMPURIFIED/ADULTERATED	MEAN MIC
1–(CITYMIXA/EC)	20.75	4.21	77.50	15.19	17.36
2-(CITYMIXB/EC)	21.00	4.40	77.50	1.61	30.56
4-(CITYMIX/EC)	19.50	NES	78.60	0.88	13.89
5-(CITYMIXTUART/WC)	18.20	4.39	81.80	4.85	50.00
6-(MANGO/WC)	16.50	4.16	82.00	0.81	30.56
9-(STRANDVELD/WC)	20.50	4.46	78.00	1.95	28.47
12-(BUSHVELD/KZN)	20.50	NES	77.50	7.61	14.58
13-(BUFFALOTHORN/NC)	17.50	4.99	81.00	2.53	25.69
14-HOOKTHORN/NC)	16.00	4.20	82.50	3.60	50.00
15-(ONION/WC)	18.00	5.09	80.25	2.34	15.28
16-(FYNBOS/WC)	18.50	NES	79.50	4.98	10.42
17-(AKMS/FS)	17.50	4.89	81.00	2.04	14.58
18-(MIXEDGUM/FS)	16.50	4.20	81.50	0.61	15.97
19-(CITYMIX/FS)	17.00	4.52	81.25	2.78	11.11
20-(FORRESTREDGUM/WC)	17.60	4.41	80.60	0.44	17.36
21-(SUGARGUM/WC)	18.80	4.45	79.00	2.11	30.56
24-(STRANDVELD/WC)	17.25	4.38	81.25	0.89	25.00
25-(BUCFYN/WC)	18.25	4.48	79.75	4.42	25.00
26-(FYNBOS/WC)	18.30	NES	80.00	0.41	20.14
27-(AEF/WC)	19.00	4.28	79.00	1.09	20.14
31-(MACADAMIA/WC)	20.50	4.28	78.00	0.19	27.78
35-(FYNBOSEC/WC)	19.00	4.49	79.25	2.69	26.39
36-(EUCLADFICI/WC)	18.70	4.35	79.50	1.24	15.97
37-(FYNBOS/WC)	18.50	4.12	79.50	2.87	26.39
38-(WASBESSFYNBOS/WC)	18.00	4.36	80.00	0.83	20.14
40-(FYNBOSGUARRI/WC)	15.80	3.89	82.50	2.30	31.94
41-(INDIGENOUS/WC)	21.60	4.22	76.75	2.70	13.19
43-(KARROVELD	19.80	4.06	78.20	3.10	21.53
44-(STRANDVELD/WC)	19.00	4.79	79.00	1.89	22.92
45-(FYNBOS/WC)	20.50	4.14	77.50	2.08	20.14
47-(SALIGNAGUM/KZN)	21.50	4.50	77.00	19.43	50.00
48-(BRE/EC)	19.00	4.32	79.00	5.50	29.17
49-(CITRUS/EC)	17.25	4.02	81.25	3.24	45.14
51-(URBANFORAGE/WC)	18.25	4.65	80.10	1.47	17.36

REFERENCE CODE	WATER CONTENT %	PH	SUGAR CONTENT %	% IMPURIFIED/ADULTERATED	MEAN MIC
52-(SUBURBANGARDEN/WC)	18.30	NES	80.10	0.41	14.58
53-(LITCHI/MP)	18.80	NES	79.40	33.60	45.14
MEAN	18.66 ± 1.51	4.39 ±0.28	79.63 ± 1.58	4.02± 6.38	

NES-Not Enough Sample for analysis

4.3.1 Water content

The moisture content of the honey samples tested ranged from 15.80-21.60 with a mean moisture content of 18.66 ± 1.51. The limit set by the Codex standard is a maximum of 20.00% as per European regulations (The Council of European Union, 2002). Of the 36 samples tested, 8 samples (22.22%) were over the maximum stipulated by the Codex. These results were comparable to the results obtained in Southern Africa (10.09%-20.73%) (Serem and Bester, 2012), Algeria 14.64%-19.04% (Ouchemoukh et al., 2010) and Morocco 14.30%-20.20% (Chakir et al., 2011). This demonstrates that moisture content is affected by climatic and geographic conditions. Nevertheless, human error during testing or condensation may have inadvertently resulted in the addition of water as well.

The degree of honey maturity as well as the ambient temperature are related to and may directly affect the moisture content. Moisture content is a key parameter in the quality analysis of honey as fermentation is a concern. Fermentation would be a factor in the determination of shelf life of a product as honey fermentation occurs during storage due to the activity of osmotolerant yeasts. This results in the formation of ethyl alcohol and carbon dioxide. Moisture content is also dependent on factors such as ripening, moisture of the nectar and harvesting. Storage conditions of honey may alter the moisture content as a result of water transfer (Gomes et al., 2010; Zamora et al., 2010; Chakir et al., 2011)

Samples with a low moisture content are considered to be of high quality (Voidaroua et al., 2011). The National Honey Board states that honeys possessing a moisture content less than 17.1% will not ferment should the honey be stored appropriately. Air with a relative humidity above 60% will allow the absorption of moisture by honey with a moisture content of 18.8% or less (Olaitan et al., 2007). Hygroscopicity is defined as the ability to retain water. This is an important factor to be considered in the processing and storage of honey. Hygroscopicity, in terms of excess water content, may hinder the stability of honey and

negatively affect its storage and preservation (Olaitan et al., 2007). It may do so as a high water content is a platform for the thriving of potential pathogens.

4.3.2 pH

The pH of the samples tested in this study ranged from 3.89-5.09, displaying acidic characteristics. The mean pH was 4.39 ± 0.28. A pH measurement could not be obtained for six samples as there was a limited quantity of sample. Honey is typically acidic, with a pH ranging between 3.2-4.5 (White, 1975). Acidic characteristics impart to honeys antimicrobial activity as a low pH inhibits and prevents microbial growth (Shenoy et al., 2012). Gluconic acid and inorganic ions in honey are components postulated to contribute to honeys acidic nature (Ouchemouk et al 2010). Thus, the pH is a beneficial guide to assessing microbial growth. Pathogens usually thrive in a pH of 7.2-7.4 (Molan, 1992). The pH range in the study was 3.89 to 5.09, and is therefore low enough to inhibit potential pathogens. These results were similar to those reported in a previous SA study where Basson and Grobler (2008) reported a pH of approximately 3.6 for the SA honey samples tested (Basson and Grobler, 2008). Serem and Bester (2012) investigated the pH of honeys from Southern Africa including SA and Mozambique and these values ranged from 3.87-5.12 (Serem and Bester, 2012). Similarly, pH values ranging from 3.50-4.43 were seen in Algeria, a completely different geographical region (Ouchemoukh et al., 2010). A study with Moroccan honeys revealed pH values of 3.80-4.50 (Malika et al., 2005). These values are similar to findings of this study. The pH of honey plays a vital role during the harvesting and storage of honey as this parameter will affect the consistency, stability and shelf life (Terrab et al., 2004).

4.3.3 Sugar content

The sugar content range was 77.00-82.50%, with a mean sugar content of 79.63±1.58%. Honey may act as a desiccant due to its hyperosmolar trait. Due to its large content of solids and low moisture content, it draws water from the pathogen thereby destroying it. Due to the supersaturated nature of honey, it leaves very few free water molecules within the compound to facilitate thriving of possible pathogens. The sugar present in honey imparts characteristics such as viscosity, hygroscopicity, granulation and energy value (Ouchemoukh et al., 2010). Sugars help honey to demonstrate antimicrobial activity by producing an osmotic effect (Bangroo et al., 2005). Sugar profiles of honey have been utilised to differentiate honey type and geographical origin, as the sugar content varies with honey type. The sugar content is dependent on the flora the bees forage on and geographical weather conditions (Olaitan et al., 2007). Sugar content range in this study was 77.00-82-50%. This is comparative to a study done on Spanish honeys where the range was

78.8%-84.00% (Terrab et al., 2004) and in Algerian honeys where similar ranges of 73.05-81.38% were observed (Ouchemouk et al., 2010).

4.3.4 Impurification

The percentage impurification ranged from 0.19-33.60, with a mean percentage ranging between 4.02±6.38%. The main constituents of honey, fructose and glucose, can be artificially added to honey to misrepresent the pure honey. Three samples [1–(CITYMIXA/EC), 47-(SALIGNAGUM/KZN) and 53-(LITCHI/MP)] displayed substantial impurification with percentages of 15.19, 19.43 and 33.60 % respectively. When compared to the mean percentage impurification of 4.02%, the values were extensive. This confirmed the lack of purity of these honey. Furthermore, there are economic ramifications of the impurification of honeys and this practise, if deliberate (adulteration), can be regarded as unethical and illegal in some countries (Fairchild, 2000).

Despite the possibility that the above-mentioned physicochemical properties may influence antimicrobial activity, no definite correlation confirming this, was observed in this study as depicted by the MIC values obtained.

4.4 Conclusions

- The moisture content of the SA honey samples tested ranged from 15.80-21.60% with a mean moisture content of 18.66 ± 1.51%.
- The pH of the samples tested in this study ranged from 3.89-5.09 with a mean pH of 4.39 ± 0.28.
- The sugar content ranged from 77.00-82.50% with a mean sugar content of 79.63 ± 1.58%.
- All 36 samples tested were to some extent impurified with the percentage impurification ranging from 0.19-33.60%, with a mean percentage of impurification of 4.02 ± 6.38%.
- In this study no correlation could be observed between the physiochemical properties and antimicrobial effects of honey despite these factors influencing antimicrobial activity in theory.

CHAPTER 5
CONCLUSION

This study was performed with the aim of validating the antimicrobial efficacy of SA honeys in wound infections. The study endeavoured to deliver insight into the potential to utilise SA honey as an effective antimicrobial agent in wound healing. Results from this study certainly highlighted that selected SA honey samples have noteworthy antimicrobial activity against pathogens associated with wound infections. Key focus areas of this research report were the antimicrobial properties of various SA honeys, their antimicrobial effects when combined with conventional antimicrobials and their physicochemical properties.

5.1 Antimicrobial activity of honey

Evaluation of the potential antimicrobial properties of the various SA honeys from varying geographical locations within the country revolved around rigorous *in vitro* testing. Extensive antimicrobial testing against a variety of common wound pathogens was conducted to determine the minimum concentration of honey that can successfully inhibit the growth of these pathogens tested in this study. Commercially available Manuka honey was utilised as a control and antimicrobial activity of these samples ranged from 15.28-41.67%. The mean MICs of the SA honey samples tested ranged from 10.42-50.00%. The greatest antimicrobial activity was exhibited by honey sample 16-(FYNBOS/WC) which displayed a mean MIC of 10.42±8.27%.

5.2 Combination studies

Combination studies were undertaken with the aim being to assess and evaluate the potential for combining honey, which is a complementary medicine, with conventional antimicrobials such as ciprofloxacin, gentamicin and antifungal agents such as amphotericin B and nystatin to investigate whether synergism, additive or antagonistic effects were observed. Honey displayed noteworthy potential to be combined with antibiotics namely; ciprofloxacin and gentamicin and antifungals namely; nystatin to produce synergism. A summary of the most synergistic interactions observed are demonstrated in Table 5.1.

Table 5.1: The ΣFIC for honey samples displaying synergistic interactions with selected allopathic antimicrobials against the respective pathogen tested.

HONEY SAMPLE	ANTIBIOTIC	PATHOGEN	ΣFIC
18-(MIXEDGUM/FS	Ciprofloxacin	*P. aeruginosa*	0.38
19-(CITYMIX/FS)	Ciprofloxacin	*P. aeruginosa*	0.50
16-(FYNBOS/WC)	Gentamicin	*S. aureus*	0.27
18-(MIXEDGUM/FS)	Gentamicin	*S. aureus*	0.27
19-(CITYMIX/FS)	Gentamicin	*S. aureus*	0.27
26-(FYNBOS/WC)	Gentamicin	*S. aureus*	0.27
41-(INDIGENOUS/WC)	Gentamicin	*S. aureus*	0.27
16-(FYNBOS/WC)	Gentamicin	*P. aeruginosa*	0.31
19-(CITYMIX/FS)	Gentamicin	*P. aeruginosa*	0.47
16-(FYNBOS/WC)	Nystatin	*C. albicans*	0.37

5.3 Physicochemical characterisation

Honey is a complex substance with many constituents which give rise to its physicochemical properties. There is limited data on the characterisation of SA honeys, and this study afforded insight into some of these properties. The pH of honey samples tested in this study ranged from 3.89 to 5.09, displaying acidic characteristics. The sugar content range was 77.00-82.50%, and the moisture content range was 15.80-21.60%. The impurification/adulteration ranged from 0.19-33.60%. Three samples namely; [1–(CITYMIXA/EC), 47-(SALIGNAGUM/KZN) and 53-(LITCHI/MP)] displayed considerable impurification.

5.4 Recommendations for future studies

The ideal standardised SA honey with the best antimicrobial activity that can be used for therapeutic purposes is the ultimate long-term goal. This could be attained if the data obtained from this study and others like this could be incorporated into a design of experiments statistical evaluation software tool (i.e. Modde or Simca-P from Umetrics) to statistically determine and predict an optimised honey sample with the most desirable antimicrobial activity. A statistical analysis catering for all variables will be an undisputed tool in determining an optimised, standardised honey.

There is a vast range of floral origin of honey. Pollen analysis should be conducted to verify botanical source of honey.

Wounds are generally polymicrobial hence a greater sample set of test pathogens should be applied. Other examples include species from *Corynebacterium, Pityrosporum, Klebsiella, Acinetobacter* and *Stenotrophomonas* etc. Similarly, fungal infections are not limited to just *Candida* species. Other problematic fungal species affecting the skin include; *Trichophyton mentagrophytes* and *Microsporum canis*are which are also important to evaluate. Hence a greater microbial and fungal sample set would need to be tested against with the optimised, standardised honey to fully elucidate its potential as an antimicrobial and anti-fungal agent.

Future combination studies should include the investigation of a greater number of antibiotic agents. A single antibiotic from each class including; penicillins, cephalosporins, carbapenams, macrolides, tetracyclines, aminoglycosides, sulphonamides and quinolones should be assessed in combination with the optimised honey samples. This will allow for a more holistic and accurate determination of honeys combination potential with conventional antibiotics, to investigate whether synergism, additive or antagonistic effects are observed to enhance and optimise the therapeutic outcome of certain infectious diseases.

There have been in *vivo* studies on the effectiveness of honey as an antimicrobial agent (El-Banby et al., 1989; Gupta et al., 1992; Suguna et al., 1992). Despite this, these studies are 20 years and older and do not include any SA honeys. Further *in-vivo* studies are required to confirm the noteworthy *in-vitro* results of SA honey and possibly form a correlation.

5.5 Final conclusion

Results from this study highlight the potential for using honey as an effective antimicrobial agent. Despite being an *in vitro* study, this study forms a platform for expanding honey-based antimicrobial treatment of certain infectious diseases, especially in South Africa. Honey as a wound dressing is of particular importance in the South African health care setting as a honey would be a favourable dressing for patients in rural areas where there is the possibility of infection occurring before first-line medical treatment is obtained. Furthermore, to date, there have been no reports of resistance to honey (Blair et al., 2009; Cooper et al., 2010). Thus, there is a need for the development of honey dressings and similar wound healing products containing honey, particularly in third world countries. The cost-effectiveness of honey as either a sole or adjunctive therapeutic agent makes it a feasible option for treating certain infections. In addition, the medicinal value attributed to selected honey samples examined here, impacts positively on the commercial value of SA honey. The value of honey with high therapeutic potential increases, as seen in the case of

Manuka honey obtained from New Zealand. Thus, this will have a significant impact on the bee keeping industry. In the event of a honey being marketed, SA bee keepers will have a competitive edge on the highly stringent global market.

REFERENCES

Abd-El, A.M., El-Hadidy, M.R., El-Mashad, N.B and El-Sebaie, A.H. 2007. Antimicrobial effect of bee honey in comparison to antibiotics on organisms isolated from infected burns. Annals of Burns and Fire Disasters. 20, 80-83.

Abdelmalek, M., Moussa, A., Noureddine, D and Saad A. 2012. Antibacterial activity of honey alone and in combination with *Nigella sativa* seeds against *Pseudomonas aeruginosa* infection. Asian Pacific Journal of Tropical Disease. 2, 428-430.

Abdelrahman, T and Newton, H. 2011. Wound dressings: Principles and practice. Surgery. 29, 491-495.

Adams, A.P., Santschii, E.M and Mellencamp, M.A. 1999. Antibacterial properties of a silver-coated nylon wound dressing. Veterinary Surgery. 28, 219-225.

Al-Jabri, A.A. 2005. Honey, milk and antibiotics. African Journal of Biotechnology. 4, 1580-1587.

Al-Jabri, A.A., Al-Hosni, S.A., Nzeako, B.C., Al-Mahrooqi, Z.H and Nsanze, H. 2005. Antibacterial activity of Omani honey alone and in combination with gentamicin. Saudi Medical Journal. 26, 767-771.

Allen, K.L., Molan, P.C and Reid, G.M. 1991. A survey of the antibacterial activity of some New Zealand honeys. Journal of Pharmacy and Pharmacoloy. 43, 817-822.

Al-Naama, R.T. 2012. Antibacterial activity of various honey types of Algeria against Pathogenic Gram-Negative Bacilli: *Escherichia coli* and *Pseudomonas aeruginosa*. Journal of Bacteriology Research. 1, 64-67.

Alvarez-Suarez, J.M., Tulipani, S., Diaz, D., Estevez, Y., Romandini, S., Giampieri, F., Damiani, E., Astolfi, P., Bompadre, S and Battino, M. 2010. Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. Food and Chemical Toxicology. 48, 2490-2499.

Alvaro-Meca, A., Jensen, J., Micheloud, D., Díaz, A., Gurbindo, D and Resino, S. 2013. Rate of candiasis among HIV-infected children in Spain in the era of highly active antiretroviral therapy 1997-2008. BMC Infectious Diseases. 13, doi: 10.1186/1471-2334-13-115.

Al-Waili, N.S. 2004. Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wounds and conjunctiva. Journal of Medicinal Food. 7, 210-222.

Anderson, R.H., Buys, B and Johannsmeier, M.F.1983. Beekeeping in South Africa. Director of Publications. Cornwell University.

Angelique, M.R and Rodeheaver, G.T. 2001. Effectiveness of a new antimicrobial gauze dressing as a bacteria barrier (pamphlet). Kendall. Wound care research and development, Mansfield, MA.

Anyanwu, C.U. 2012. Investigation of *in vitro* antifungal activity of honey. Journal of Medicinal Plants Research. 6, 3512-3516.

Ashley, E.S.D., Lewis, R., Lewis, J.S., Martin, C and Andes, D. 2006. Pharmacology of systemic antifungal agents. Clinical Infectious Diseases. 43, 28-39.

Ayaad, T.H., Shaker, G.H and Almuhnaa, A.M. 2011. Isolation of antimicrobial peptides from Apis florae and Apis carnica in Saudi Arabia and investigation of the antimicrobial properties of natural honey samples. Journal of King Saud – Science. 24, 193-200.

Bangroo, A.K., Khatri, R and Chauhan, S. 2005. Honey dressing in paediatric burns. Journal of Indian Association of Paediatric Surgeons. 10, 172-175.

Basson, N.J and Grobler, S.R. 2008. Antimicrobial activity of two South African honeys produced from indigenous *Leucospermum cordifolium* and *Erica* species on selected micro-organisms. BMC Complementary and Alternative Medicine. 8, Doi: 10.1186/1472-6882-8-41.

Basualdo, C., Sgroy, V., Finola, M.S and Marioli, J.M. 2007. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Veterinary Microbiology. 124, 375-381.

Blair, S.E., Cokcetin, N.N., Harry, E.J and Carter, D.A. 2009. The unusual antibacterial activity of medical-grade *Leptospermum* honey: antibacterial spectrum, resistance and transcription analysis. European Journal of Clinical Microbiology and Infection. 28, 1199-1208.

Bowler, P.G., Duerden, B.I and Armstrong, D.G. 2001. Wound microbiology and associated approaches to wound management. Clinical Microbiology Reviews. 14, 244-269.

Brady, N., Molan, P and Bang, L. 2004. A survey of non-Manuka New Zealand honeys for antibacterial and antifungal activities. Journal of Apicultural Research. 43, 47-52.

Bray, R., Caskey, P.R and Patel, C. 2009. Wound dressings. European Patent EP1450871B1. PCT/GB2002/005441.

Brudzynski, K and Lannigan, R. 2012. Mechanism of honey bacteriostatic action against MRSA and VRE involves hydroxyl radicals generated from honeys hydrogen peroxide. Frontiers in Microbiology. 3, 1-8.

Chakir, A., Romane, A and Marcazzan, G.L. 2011. Physicochemical properties of some honeys produced from different plants in Morocco. Arabian Journal of Chemistry. doi:10.1016/j.arabjc.2011.10.013.

Chesson, L.A., Tipple, B.J., Erkkila, B.R., Cerling, T.E and Ehleringer, J.R. 2010. B-Hive: Beeswax hydrogen isotopes as validation of environment. Part I: Bulk honey and honey comb stable isotope analysis. Food Chemistry. 125, 576-581.

Chusri, S., Sompetch, K., Mukdee, S., Jansrisewangwong, S., Srichai, T., Maneenoon, K., Limsuwan, S and Voravuthikunchai, S.P. 2012. Inhibition of *Staphylococcus epidermidis,* Biofilm formation by traditional Thai herbal recipes used for wound treatment. Evidence-Based Complementary and Alternative Medicine. doi:10.1155/2012/159797.

Cooper, R. 1999. The use of honey as an antiseptic in managing *Pseudomonas* infections. Journal of Wound Care. 8, 161-164.

Cooper, R.A., Wheat, E., Burton, N.F. 2008. An investigation into the wound healing properties of Welsh honeys**.** Journal of Apicultural Research and Bee World*.* 47, 251-255.

Cooper, R.A and Jenkins, L. 2009. A comparison between medical grade honey and table honeys in relation to antimicrobial efficacy. Wounds. 21, 29-36.

Cooper, R.A., Jenkins, L., Henriques, A.F., Duggan, R.S and Burton, N.F. 2010. Absence of bacterial resistance to medical-grade Manuka honey. European Journal of Clinical Microbiology and Infectious Diseases. 29, 1237-1241.

Cooper, R.A., Molan, P.C and Harding, K.G. 1999. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. Journal of the Royal Society of Medicine. 92, 283-285.

Cooper, R., 2007. Honey in wound care: antibacterial properties, GMS KrankenhaushygieneInterdisziplinar. 2, 1863-5245.

Cooper, R.A., Halas, E and Molan, P.C. 2002. The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. Journal of Burn Care and Rehabilitation. 23, 366-370.

Cooper, R.A., Wheat, E and Burton, N.F. 2008. An investigation into the wound healing properties of Welsh honeys. Journal of Apicultural Research and Bee World. 47, 251-255.

Darwish R.M and Aburjai, T.A. 2010. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escheria coli*. BMC Complementary and Alternative Medicine.10, 1-8.

David, M.Z. and Daum, R.S. 2010. Community-associated methicillin-resistant *Staphylococcal aureus*: Epidemiology and clinical consequences of an emerging epidemic. Clinical Microbiology Reviews. 23, 616-687.

Debas, H.T., Laxminarayan, R and Straus, S.E. 2006. Complementary and Alternative Medicine. Lifestyle, Chapter 69. World Bank. Washington.

Demera, J.H and Angert, E.R. 2004. Comparison of the antimicrobial activity of honey produced by *Tetragonis caangustula* (Meliponinae) and Apismellifera from different phytogeographic regions of Costa Rica. Apidologie. 35, 411-417.

Department of Water and Affairs and Forestry. 2005. The honey Trade. Forestry Sub-Sector Studies.

El-Banby, M., Kandil, A., Abou-Sehly, G., El-Sherif, M.E and Abdel-Wahed, K. 1989. Healing effect of floral honey and honey from sugar-fed bees on surgical wounds (animal model).Fourth International Conference on Apiculture in Tropical Climates. Cairo: International Bee Research Association, London, 46-49.

Eloff, J.N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts of bacteria. Planta Medica. 64, 711-713.

Estevinho, L., Pereira, A.P., Moreira, L., Dias, L.G and Pereira, E. 2008. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food and Chemical Toxicology. 46, 3774-3779.

Fairchild, G.F., Capps Jr, O and Nichols, J.P. 2000. Impacts of Economic Adulteration of the U.S. Honey Industry. Presented at the Western Agricultural Economics Association Annual Meetings, Vancouver, British Columbia. June 29-July 1.

Fouda, M.G., Wittke, R., Knittel, D and Schollmeyer, E. 2009. Use of chitosan/polyamine biopolymers based cotton as a model system to prepare antimicrobial wound dressings. International Journal of Diabetes Mellitus. 1, 61-64.

French, V.M., Cooper, R.A and Molan, P.C. 2005. The antibacterial activity of honey against coagulase-negative *Staphylococci*. Journal of Antimicrobial Chemotherapy. 56, 228-231.

Frost, M.R., Jackson, S.W and Stevens, P.J. 1980. Adsorption of bacteria onto activated charcoal cloth: An effect of potential importance in the treatment of infected wounds. Microbios Letters. 13, 135-140.

Gallardo-Chacon, J., Caselles, M., Izquierdo-Pulido, M and Rius, N. 2008. Inhibitory activity of monofloral and multifloral honeys against bacterial pathogens. Journal of Apicultural Research and Bee World. 47, 131-136.

Goldblatt, P and Manning, J.C. 2002. Plant diversity of the cape region of South Africa. Annals of the Missouri Botanical Garden. 89, 281-302.

Gomes, S., Diaz, L.G., Moreira, L.L., Rodrigues, P and Estevinho, L. 2010. Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food and Chemical Toxicology. 48, 544-548.
Grothier, l and Cooper, R. 2011. Medihoney^{TM} Dressings. WoundsUK. 6, 1-6.

Guillemot, D. 1999. Antibiotic use in humans and bacterial resistance. Current Opinion in Microbiology. 2, 494-498.

Gulfraz, M., Iftikhar, F., Raja, S., Asif, S., Mehmood, S., Anwar, Z and Kaukob, G. 2010. Quality assessment and antimicrobial activity of various honey types of Pakistan. African Journal of Biotechnology. 41, 6902-6906.

Guo-xue, W., Ji-kun, H and Jun, Y. 2011. Honey safety standards and its impact on China's honey export. Journal of Integrative Agriculture. 11, 684-693.

Gupta, S.K., Sing, H., Varshney, A.C and Prakash, P. 1992. Therapeutic efficacy of honey in infected wounds in buffalos. Indian Journal of Animal Sciences. 62, 521-523.

Hamouda, H.M and Marzouk, D.S. 2011. Antibacterial activity of Egyptian honey from different sources. International Journal of Microbiological Research. 2, 149-155.

Hemaiswarya, S., Kruthiventi, A.K and Doble, M. 2008. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine.15, 639-652.

Henriques, A., Burton, N.F and Cooper, R.A. 2005. Antibacterial activity of selected Portuguese honeys. Journal of Apicultural Research.44,119-123.

Henriques, A.F., Jenkins, R.E. Burton, N.F and Cooper R.A. 2010. The intracellular effects of Manuka honey on *Staphylococcus aureus*. European Journal of Clinical Microbiology and Infectious Diseases. 29, 45-50.

Honardoust, D. 2012. The Canadian Association of Medical Spas and Aesthetic Surgeons. http://www.camacs.ca/Directors.php, accessed 20th December 2012.

Hotoson-Moore, A. 2003. Use of topical povidone–iodine dressings in the management of mycotic rhinitis in three dogs. Journal of Small Animal Practice. 44, 326-329.

Irish, J., Blair, S. and Carter, D.A. 2011. The antibacterial activity of honey derived from Australian flora. Antibacterial Honey from Australian Plants. 6. Doi: 10.1371/journal.pone.0018229.

Jayaraman, P., Sakharkar, M.K and Sakharkar, K.R. 2010. Activity and interactions of antibiotic and phytochemical combinations against *Pseudomonas aeruginosa in vitro*. International Journal of Biological Sciences. 6, 556-568.

Jenkins, R., Burton, N and Cooper R. 2011. Manuka honey inhibits cell division in methicillin-resistant *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy. 66, 2536-2544.

Jenkins, R.E and Cooper, R. 2012. Synergy between oxacillin and Manuka honey sensitizes methicillin-resistant *Staphylococcus aureus* to oxacillin. Journal of Antimicrobial Chemotherapy. 67, 1405-1407.

Jenkins, R.E and Cooper, R. 2012. Improving antibiotic activity against wound pathogens with manuka honey *in vitro*. 7, doi:10.1371/journal.pone.0045600

Karakoc, B and Gerceker, A.A. 2001. *In-vitro* activities of various antibiotics, alone and in combination with amikacin against *Pseudomonas aeruginosa*. International Journal of Antimicrobial Agents. 18, 567-570.

Karayil, S., Deshpande, S.D and Koppikar, G.V. 1998. Effect of honey on multidrug resistant organisms and its synergistic action with three common antibiotics. Journal of Postgraduate Medicine. 44, 93-96.

Karousou, R., Koureas, D.N and Kokkini, S. 2005. Essential oil composition is related to the natural habitats: *Coridothymus capitus* and *Satureja thymbra* in NATURA 2000 sites of Crete. Phytochemistry. 66, 2668-2673.

Kaskoniene, V and Venskutonis, P.R. 2010. Floral markers in honey of various botanical and geographical origins: a review. Comprehensive Reviews in Food Science and Food Safety.9, 620.

Khoo, Y., Halim, A.S., Singh, K.K and Mohamad N. 2010. Wound contraction effects and antibacterial properties of Tualang honey on full thickness burn wounds in rats in comparison

to hydrofibre. BMC Complementary and Alternative Medicine. 10, Doi: 10.1186/1472-6882-10-48.

Khosravi, A.R., Shokri, H., Katiraee, F., Ziglari, T and Forsi, M. 2008. Fungicidal potential of different Iranian honeys against some pathogenic *Candida* species. Journal of Apicultural Research and Bee World. 47, 256-260.

Knill, C.J., Kennedy, J.F., Mistry, J., Miraftab, M., Smart, G., Groocock, M.R and Williams, H.J. 2004. Alginate fibres modified with unhydrolysed and hydrolysed chitosans for wound dressings. Carbohydrate Polymers. 55, 65-76.

Kronda, J.M., Cooper, R.A and Maddocks, S.E. 2013. Manuka honey inhibits siderophore production in *Pseudomonas aeruginosa*. Journal of Applied Microbiology. 115, 86-90.

Kumari, S., Harjai, K and Chhibber, S. 2009. Topical treatment of *Klebsiella pneumoniae* B5055 induced burn wound infection in mice using natural products. Journal of Infection in Developing Countries. 6, 367-377.

Kwakman, P.H.S and Zaat, S.A.J. 2012. Antibacterial components of honey. International Union of Biochemistry and Molecular Biology Life. 48-55.

Lambert, P.A. 2002. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa.* Journal of the Royal Society of Medicine. 95, 22-26.

Lawrence, J.C. 1994. Dressings and wound infection. American Journal of Surgery. 167, 21-24.

Letsch, S.W., Lazenby, H.C., Levit, K.R and Cowan, C.A. 1991. National health expenditures. Health care financing. 14, 1-30.

Limoncu, M.H., Ermertcan, S., Cetin, C.B., Cosa, G and Dinc, G. 2003. Emergence of phenoytypic resistance to ciprofloxacin and levofloxacin in methicillin-resistant and methicillin-sensitive *Staphylococcal aureus* strains. International Journal of Antimicrobial Agents. 21, 420-424.

Lusby, P.E., Coombes, A and Wilkinson, J.M. 2002. Honey: A potent agent for wound healing? Wound Ostomy and Continence Nurses Society. 29, 255-300.

Lusby, P.E., Coombes, A and Wilkinson, J.M. 2005. Bactericidal activity of different honeys against pathogenic bacteria. Archives of Medical Research. 36, 464-467.

Maeda, Y., Loughrey, A., Earle, J.A., Millar, B.C., Rao, J.R., Kearns, A., McConville, O., Goldsmith, C.E., Rooney, P.J., Dooley, J.S., Lowery, C.J., Snelling, W.J., McMahon, A., McDowell D and Moore J.E. 2008. Antibacterial activity of honey against community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Complementary Therapies in Clinical Practice. 14, 77-82.

Malika, N., Mohammed, F and Chakib, E. 2005. Microbiological and physico-chemical properties of Moroccan honey. International Journal of Agriculture Biology. 5, 773-776.

Mandal, S., DebMandal, M., Kumar, N and Saha, K. 2010. Antibacterial activity of honey against clinical isolates of *Escherichia* coli, Pseudomonas *aeruginosa*and *Salmonella enterica serovar typhi*. Asian Pacific Journal of Tropical Medicine. 3, 961-964.

Manyi-loh, C.E., Clark, A.M., Munzhelele, T., Green, E., Mkwetshana, N.F and Ndip,, R.N. 2010. Selected South African honeys and their extracts possess *in-vitro* anti-*helicobacter pylori* activity. Archives of Medical Research. 41, 324-331.

Manyi-loh, C.E., Clark, A.M and Ndip,, R.N. 2012. Detection of phytoconstituents in column fractions of n-hexane extract of Goldcrest honey exhibiting anti-*Helicobacter pylori* activity. Archives of Medical Research. 43, 197-204.

Mathews, K.A and Binnington, A.G. 2002. Wound management using honey. Compendium. 24, 53-60.

Mavric, E., Wittmann, S., Barth, G and Henle, T. 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. Molecular Nutrition and Food Research. 54, 483 - 489.

Meda, A., Lamien, C.E., Millogo, J., Romito, M. and Nacoulma, O.G. 2004. Therapeutic uses of honey and honeybee larvae in central Burkina Faso. Journal of Ethnopharmacology. 95, 103-107.

Mehryar, L and Esmaiili, M. 2011. Honey and Honey Adulteration Detection: A Review, 11th International Congress on Engineering and Food, Athens, Greece.

Meletiadis, J., Pournaras, S and Walsh, T.J. 2010. Defining fractional inhibitory concentration index for additive interactions based on self-drug additive combinations, Monte Carlo simulation analysis, and *in vitro-in vivo* correlation data for antifungal drug combinations against *Aspergillus fumigates*. Antimicrobial Agents and Chemotherapy. 54, 602-609.

Molan, P.C. 1992. The antibacterial activity of honey 1, The nature of antibacterial activity. Bee World. 73, 59-76.

Molan, P.C. (a). 1999. The role of honey in the management of wounds. Journal of Wound Care. 8, 415-418.

Molan, P.C. (b). 1999. Why honey is effective as a medicine. 1. Its use in modern medicine. Bee World.80, 80-92.

Molan, P.C. 2001. Honey as a topical antibacterial agent for treatment of infected wounds. World Wide Wounds.*www.worldwidewounds.com/2001/.../Molan/honey-as-topical-agent.htm,* accessed 3/14/2011.

Molan, P.C. 2002. Re-introducing Honey in the Management of Wounds and Ulcer-Theory and Practice. Ostomy/Wound Management. 48, 28-40.

Molan P.C. 2006. The evidence supporting the use of honey as a wound dressing. International Journal of Lower Extremity Wounds.5, 40-54.

Moore K., Thomas A and Harding K.G. 1997. Iodine released from the wound dressing Iodosorb modulates the secretion of cytokines by human macrophages responding to bacterial lipopolysaccharide. International Journal of Biochemistry and Cell Biology. 29, 163-171.

Moussa, A., Noureddine, D., Mohamed, H.S., Abdelmelek, M.S and Saad, A. 2012. Antibacterial activity of various honey types of Algeria against *Staphylococcus aureus* and *Streptococcus pyogenes*. Asian Journal of Tropical Medicine. 5, 773-776.

Mullai, V and Menton, T. 2005. Antibacterial activity of honey against Pseudomonas aeruginosa. The Journal of Alternative and Complementary Medicine. 13, 439-442.

Muller, P., Alber, D.G., Turnbull, L., Schlothauer, R.C., Carter, D.A., Whitchurch, C.B and Harry, E.J. 2013. Synergism between Medihoney and Rifampicin against methicillin-resistant *Staphylococcus aureus*. 8, doi:10.1371/journal.pone.0057679.

Munoz, P., Guinea, J., Rojas, L and Bouza, E. 2010. New antifungal agents for the treatment of candidaemia. International Journal of Antimicrobial Agents. 36, doi:10.1016/j.ijantimicag.2010.11.007.

Nasser, S., Mabrouk, A and Maher, A. 2003. Colonisation of burn wounds in Ain Shams University burn unit. Burns. 29, 229-233.

National Committee for Clinical Laboratory Standards. 2003. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved standard, 6th edition. USA.

Nyasulu, P., Murray, J., Perovic, O and Koornhof, H. 2012. Antimicrobial resistance surveillance among nosocomial pathogens in South Africa: Systematic review of published literature. Journal of Experimental and Clinical Medicine. 4, 8-13.

Olaitan, P.B., Adeleke, O.E and Ola, I.O. 2007. Honey a reservoir for micro-organisms and an inhibitory agent for microbes. African Health Sciences. 7, 159-165.

Osho, A and Bello, O.O. 2010. Antimicrobial effect of honey produced by Apis mellifera on some common human pathogens. Journal of Experimental Biological Science. 1, 875-880.

Ouchemoukh, S., Schweitzer, P., Bey, M.B and Djoudad-Kadji, H. 2010. HPLC sugar profiles of Algerian honeys. Food Chemistry. 121, 561-568.

Ovington, L.G and Eisenbud, D. 2004. Dressings and cleansing agents. In: Krasner, D.L., Rodeheaver, G.T., Sibbald, R.G., eds. Chronic wound care: a clinical source book for healthcare professionals. 3rd edition. HMP communications Wayne, PA:117-28.

Patel, S. 2007. The impact of MRSA on wound healing. Wounds Essential. 2, 144-148.

Raad, I., Alrahwan, A and Rolston, K. 1998. *Staphylococcal epidermidis*: emerging resistance and need for alternate agents. Clinical Infectious Diseases. 26, 1182-1187.

Ranjbar, R., Owlia, P., Saderi, H and Mansouri, S. 2011. Patients hospitalized in a major burn center in Tehran, Iran. Acta Medica Iranica. 49, 675-679.

Serem, J.C and Bester, M.J. 2012. Physicochemical properties, antioxidant activity and cellular protective effects of honeys from southern Africa. Food Chemistry. 133, 1544-1550.

Shenoy,V.P., Ballal, M., Shivananda, P.G and Bairy, I. 2012. Honey as an antimicrobial agent against Pseudomonas aeruginosa isolated from infected wounds. Journal of Global Infectious Diseases. 4, 102-105.

Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S and Humphreys, H. 2010. Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. BMC Complementary and Alternative Medicine. 47, Doi:10.1186/1472-6882-10-47.

Simon, A., Traynor, K., Santos, K., Blaser, G., Bode, U and Molan, P. 2009. Medical honey for wound care – still the 'latest resort? Evidence-based and Complementary Medicine. 6, 165-173.

Somal, N. A., Coley, K-E., Molan, P. C and Hancock, B. M. 1994. Susceptibility of Helicobacter pylori to the antibacterial activity of Manuka honey. Journal of the Royal Society of Medicine. 87, 9-12.

South African Medicines Formulary (SAMF). 2012. The Division of Clinical Pharmacology, Faculty of Health Sciences, University of Cape Town.

Speert, D.P. 2002. Molecular epidemiology of *Pseudomonas aeruginosa.* Frontiers in Bioscience. 7, 354-361.

Stashak T.D., Farstvedt E and Othic A. Update on Wound Dressings: Indications and Best Use. Clinical Techniques in Equine Practice. 3, 148-163.

Steffansen, B and Herping, S.P.K. 2008. Novel wound models for characterising ibuprofen release from foam dressings. International Journal of Pharmaceutics. 364, 150-155.

Stemberger, A., Grimm H., Bader F., et al. 1997. Local treatment of bone and soft tissue infections with collagen gentamicin sponge. European Journal of Surgery. 578, 17- 26.

Suguna, L., Chandrakasan, G and Thomas, J.K. 1992. Influence of honey on collagen metabolism during wound healing in rats. Journal of Clinical Biochemistry and Nutrition. 13, 7-12.

Summerhays, G.E.S. 2000. Treatment of traumatically induced synovial sepsis in horses with gentamicin-impregnated collagen sponges. Veterinary Record. 147, 184-188.

Tan, H.T., Rahman, R.A., Gan, S.H., Halim, A.S., Hassan, S.A., Sulaiman, S.A and Kirnpal-Kaur, B.S. 2009. The antibacterial properties of Malaysian Tualang honey against wound and enteric microorganisms in comparison to Manuka honey. BMC Complementary and Alternative Medicine. 9, doi:10.1186,1472-6882-9-34.

Terrab, A., Recamales, A.F., Hernanz, D. 2004. Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. Food chemistry. 88, 537-542.

The Council of the European Union. 2002. Council Directive 2001/110EC of 20 December 2001 relating to honey. Official Journal of the European Communities.10, 47-52.

Theunissen, F., Grobler, S and Gedalia, I. 2001.The antifungal action of three South African honeys on *Candida albicans*. Apidologie. 32, 371-379.

Thu, H., Zulfakar, M.H and Ng, S. 2012. Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing. International Journal of Pharmaceutics. 434, 375-383.

Turner, T.D. 1979. Hospital usage of absorbent dressings. Pharmaceutical Journal. 222, 421-424.

Valencia, I.C., Kirsner, R.S and Kerdel, F.A. 2004. Microbiologic evaluation of skin wounds: alarming trend toward antibiotic resistance in an inpatient dermatology service during a 10 year period. Journal of the American Academy of Dermatology. 50, 845- 849.

Van Vuuren, S.F. 2008. Antimicrobial activity of South African medicinal plants. Journal of Ethnopharmacology. 119, 462-472.

Van Vuuren, S.F and Viljoen, A. 2011. Plant based antimicrobial studies – Methods and approaches to study the interactions between natural products. Journal of Medicinal Plant and Natural Product Research. 77, 1168-1182.

Van Wyk, B.E., Van Oudtshoorn, B and Gericke, N. 2009. Medicinal Plants of South Africa, second ed. Briza, Pretoria, South Africa.

Vázquez-González, D., Perusquía-Ortiz, A.M., Hundeiker, M and Bonifaz, A. 2013. Opportunistic yeast infections: candiasis, cryptococcosis, trichosporonosis and geotrichosis. Journal der Deutschen Dermotologischen Gesellschaft. 11, 381-394.

Visavadia, B.G., Honeysett, J and Danford, M.H. 2006. Manuka honey dressing: An effective treatment for chronic wound infections. British Journal of Oral and Maxillofacial Surgery. 46, 55-56.

Voidaroua, C., Alexopoulosb, A., Plessasb, S., Karapanouc., A., Mantzouranib, I., Stavropouloud, E., Fotoua, K., Tzoraa, A., Skoufosa, I and Bezirtzoglou, E. 2011. Antibacterial activity of different honeys against pathogenic bacteria. Anaerobe. 17, 375-379.

Vorlova, L., Karpiskova, R., Chabiniokovai, I., Kalabovak, K and Brazdova, Z. 2005. The antimicrobial activity of honeys produced in the Czech Republic. Czech Journal of Animal Science. 50, 376-384.

Wang, T., Zhu, X., Xue, X and Wu, D. 2012. Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings. Carbohydrate Polymers. 88, 75–83.

Wang, J and Li, Q.X. 2011. Chemical Composition, characterisation and differentiation of honey botanical and geographic origins. Advances in Food Nutrition Research. 62, 89-137.

White, J.W., 1975. Physical characteristics of honey. In: Crane E (ed) Honey: A comprehensive survey. Heinemann, London. 157-206.

White, J., W and Winters, K. 1989. Honey protein as internal standard for stable carbonisotope ratio detection of adulteration honey. Journal of the Association of Official Analytical Chemists. 72, 907-911.

WHO Infectious Diseases Report. 1999. Chapter 2.www.who.int/infectious-disease-report/pages/ch2text.html, accessed 5th June 2012.

Wright, G.D. 2010. Antibiotic resistance in the environment: a link to the clinic? Current Opinion in Microbiology.13, 589-594.

www.mountainside-medical.com/products/Medihoney-Honeycolloid-Dressing-with-Adhesive-Border-10%7B47%7Dbox.html, accessed 20th January 2013.

Wysocki, A.B. 2002. Evaluating and managing open skin wounds: colonization versus infection. AACN Clinical Issues. 13, 382-97.

Yousof, N., Hafiza, A. H. A., Zohdi, R. M and Bakar, M. Z. A. 2007. Development of honey hydrogel dressing for enhanced wound healing. Radiation Physics and Chemistry. 76, 1767-1770.

Zahedi, P., Rezaeian, I., Ranaei-Siadat, S., Jafari, S and Supaphol, P. 2010. A review on wound dressings with emphasis on electrospun nanpfibrous polymeric bandages. Polymers Advanced Technologies. 21, 77-95.

Zamora, M.C., Chirife, J and Roldan, D. 2010. On the nature of the relationship between water activity and % moisture in honey. Food Control. 17. doi: 10.1016/j.foodcont.2005.04.002.

APPENDIX A

Antimicrobial properties and isotope investigations of South African honeys

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Abstract

Background: The therapeutic potential of honey for the treatment of wound infections is well documented. However, the difference in antimicrobial potency among various honey types can be as large as 100-fold, depending on its geographical and botanical sources. South African (SA) honey has been poorly explored as an antimicrobial agent and given the well-established antimicrobial properties of the indigenous plant species from SA, there is the potential that honey from this geographical region may exhibit noteworthy anti-infective properties. In this study, the antimicrobial properties of 42 honey samples from eight provinces in SA (Eastern Cape, Western Cape, Northern Cape, Kwa-Zulu Natal, Free State, Mpumalanga, North West and Limpopo) were determined.

Methods: The agar dilution method was used to quantify the minimum inhibitory concentration (MIC) against various *Staphylococcus aureus* strains, *Pseudomonas aeruginosa* and two *Candida albicans* trains. In addition, the physicochemical properties including pH and water content were analysed and Stable Isotope Analysis (SIA) was performed.

Results: The study demonstrated that some SA honeys exhibit considerable antimicrobial activity. *Pseudomonas aeruginosa* was generally the most sensitive of the pathogens tested, while *C. albicans* was the least sensitive. The MICs of the honeys ranged from 6.25% to 50.00%. Samples 4-(CITYMIX/WC), 12-(BUSHVELD/KZN), 15-(ONION/WC), 16-(FYNBOS/WC), 17-(AKMS/FS), 19-(CITYMIX/FS), 41-(INDIGENOUS/WC) and 52-(SURBURBANGARDEN/WC) displayed broad-spectrum antimicrobial activity equivalent to or better than the commercially obtained manuka honey. The pH of the honeys ranged between 3.89 and 5.09, providing some validation for its antimicrobial efficacies. Stable isotope analysis (SIA) revealed strong overall trends between protein concentration and MIC suggesting close links with antimicrobial activity.

Conclusions: The future of South Africa's market for medical grade and therapeutic honeys looks promising, as there is minimal impact of sugar adulteration and the antimicrobial properties of the honeys have some superior activity.

Keywords: Wound pathogens; Minimum inhibitory concentration; Agar dilution method; Isotope analysis; Protein; Honey; Antimicrobial.

APPPENDIX B

University
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Human Research Ethics Committee (Medical)
(formerly Committee for Research on Human Subjects (Medical)

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Ref: W-CJ-120210-3

10/02/2012

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: **Prof S van Vuuren, Ms F Khan (Student No 0204087A)**

Project title: **Antimicrobial properties of South African honeys against pathogens associated with wound infections.**

Reason: This is a wholly laboratory study of honey antimicrobial properties. No human participants are involved.

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON-JONES
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2012 -02- 10
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Professor Peter Cleaton-Jones
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