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The Use of Australian Honey in Moist Wound Management

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The Use of Australian Honey in Moist Wound Management

A report for the Rural Industries Research and Development Corporation

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Foreword

The use of honey as a therapeutic agent dates to ancient times. More recently, there has been growing interest in this 'natural' remedy, which has lead to legitimate scientific investigations. Research in New Zealand has shown that Manuka honey has very special healing properties. This honey has been described to contain "the best natural antibiotic in the World". There is much evidence to suggest that the antibacterial activity of honey is due to more than the sugars present therein. Two major sources have been shown to be responsible for the anti-microbial activity - hydrogen peroxide (from the bee) and uncharacterised chemical substances (from the floral source). Using honeys sourced from around Australia and screened against several reference microorganisms, the anti-microbial potency of one particular Australian honey was shown to be comparable to NZ Manuka honey. Initial chemical comparison also confirmed that the NZ Manuka and the "active" Australian honey are very similar. This is not unexpected since both of these remarkable honeys are derived from *Leptospermum* trees.

This project was undertaken to add value to the existing honey resource by developing products with therapeutic benefit (*i.e.* for the treatment and management of moist wounds such as burns and ulcers). The production of such valuable honeys requires the honey to be collected and processed under prescribed conditions. This involves the identification of the appropriate floral sources, the development of procedures for harvesting and handling, the evaluation of the "active" agent(s), and the registration of honey as a therapeutic agent.

In an Australia-wide context, the honey and pollen industries are estimated to be worth in the order of $A32 M (which does noes not include the value of incidental pollination of many agricultural crops). This project has significant potential to add value to the Australian Honey Industry. The value of honey sales to the New Zealand Honey Industry has increased significantly with the research and associated promotion of their native Manuka honey. The research undertaken in this project and extension of these results has promoted the use of honey for the treatment of bacterial infections associated with such injuries as burns and ulcers.

This project was funded from industry revenue which is matched by funds provided by the Australian Government.

This report is an addition to RIRDC's diverse range of over 1500 research publications. It forms part of our Honeybee R&D sub-program which aims to improve the productivity and profitability of the Australian beekeeping industry.

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Peter O'Brien
Managing Director
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Abbreviations

ATCC	American Type Culture Collection
cfu	Colony Forming Unit
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
kGy	kiloGray
mL	millilitre
NCTC	National Type Culture Collection
nm	nanometres
pH	acid scale
TGA	Therapeutic Good Administration
w/v	weight to volume

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Executive Summary

The use of honey as a therapeutic agent dates to ancient times. More recently, there has been growing interest in this 'natural' remedy, which has lead to legitimate scientific investigations. Research has shown that particular honeys have very special healing properties. One group of honeys (*Leptospermum* honeys) have been described as "the best natural antibiotic in the World".

Honeys have been shown to be active against a diverse range of microorganisms and reports of the inhibitory effect of honey on specific microorganisms are numerous. Honey has been shown to be effective against both Gram positive and Gram negative organisms, aerobic and anaerobic bacteria, as well as inhibiting spore germination of *Bacillus cereus*. The sensitivity of different bacterial species and strains to honey is extremely variable. Honey has also recently been shown to have an inhibitory effect against antibiotic resistant strains (*e.g.* golden *Staph*), which are frequently responsible for post-operative wound infection in immunologically compromised patients.

Much research effort has centred on establishing the properties of honey to which its antibacterial activity may be attributed. Such factors as osmolarity, acidity, hydrogen peroxide content and chemical components of honey have all been considered to contribute to the inhibition of bacterial growth. Honey is a highly saturated sugar solution which could suggest that this characteristic of honey would convey an antimicrobial effect. The high concentration of sugars leaves very little available water for the growth of microorganisms. However, the osmolarity of honey does not appear to be a major factor. The acidity of honey has also been suggested to explain the antibacterial activity of honey. Honey contains many organic acids, predominantly gluconic acid produced from glucose by glucose oxidase, and is characteristically acidic with pH 3.2 to 4.5. Although such an acidity level would be inhibitory to the growth of most bacterial species, there appears to be no correlation between antibacterial activity and the acidity of the honey. There is much evidence to suggest that the antibacterial activity of honey is due to more than the sugars present therein. Two major sources have been shown to be responsible for the anti-microbial activity - hydrogen peroxide (from the bee) and uncharacterised chemical substances (from the floral source). Hydrogen peroxide is known to have antimicrobial properties and can be removed efficiently by the addition of catalase to the honey prior to testing for antibacterial activity.

This project has investigated the correlation between antimicrobial activity and the non-peroxide activity of particular *Leptospermum*-derived honeys. Initial investigations tested the efficacy of these honeys against one particular bacteria (*Staphylococcus aureus*), while later studies assessed the effect of this honey on a range of food pathogens, animal pathogens (*e.g.* in mastitis) and human pathogens (*e.g.* golden *Staph*). Using honeys sourced from around Australia and screened against several reference microorganisms, the anti-microbial potency of one particular Australian honey (from *Leptospermum polygalifolium*) was shown to be comparable to NZ Manuka honey (from *L.scoparium*). The results of this screening of active honeys against pathogenic bacteria has supported the registration of honey by Capilano Honey Limited as a "Drug" with the Therapeutic Good Administration based on its antimicrobial activity.

This project was undertaken to add value to the existing honey resource by developing products with therapeutic benefit (*i.e.* for the treatment and management of moist wounds such as leg ulcers). Specific geographic region(s) in Australia which produce "active" honey have been identified, the potency of the *Leptospermum*-derived honeys against a range of bacteria has been defined, the particular chemical characteristics of these honeys have been examined, and the honey has been registered as a "Drug" with the Therapeutic Good Administration. Work is continuing to better define the specific agents responsible for the antimicrobial activity (which have been elusive to date) and to evaluate the opportunity for therapeutic benefit from honey beyond its antimicrobial activity (*i.e.* its direct wound healing benefit).

Introduction

Honey has a long and interesting history. In addition to food use, honey has been used in medicine as a dressing for wounds and inflammations, both internal and external. Recently, the medicinal use of honey has been rediscovered by the medical profession and is gaining acceptance as an antibacterial agent for treating ulcers, wounds and other surface infections. Honey is effective in rapidly clearing infection and promoting healing. The literature reports that honey has been successfully used on infections not responding to standard antiseptic and antibiotic therapy. Thus, as the number of antibiotic-resistant microbes continues to increase, the full potential of honey as a therapeutic agent will be realised.

Since Biblical times and before, honey has been known to have beneficial health effects. Honey has been used in medicine throughout recorded history and is still widely used in 'folk medicine' (Majno, 1975). The ancient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey in the treatment of wounds and intestinal disorders (Zumla and Lulat, 1989). The 'Smith papyrus', an ancient Egyptian text (dating between 2600 and 2200 BC), prescribes a mixture of mrhy (grease), byt (honey) and ftt (vegetable fibre), as a standard wound salve (as translated from hieroglyphic symbols, Majno, 1975). The Roman scientist Pliny (23-79 AD) recommended honey for abscesses of the mouth and combined honey with the fat of fish to treat wounds (Yoirish, 1977).

An extensive review of the antibacterial properties of honey, as then understood, was published in 1966 (White, 1966) and knowledge has since been comprehensively updated in a more recent review by Molan (1992a,b). Over recent times, there has been a large amount of research into the antibacterial nature of honeys and the effectiveness of honey in assisting the healing process. Experimentally, Bergman *et al.* (1983) provided evidence suggesting that honey accelerates wound healing in mice. There have been many reports of the beneficial effects of honey used as a topical treatment for a wide range of wounds, ulcers and abscesses (Cavanagh *et al*., 1970; Blomfield, 1973; Armon, 1980; Farouk *et al*., 1988; Efem, 1992; Ndayisaba *et al*., 1992; Phuapradit and Saropala, 1992).

Efem (1988) reported that infected wounds and ulcers became sterile within one week of topical application of honey. In a recent report, application of honey has successfully been used to manage Fourier's gangrene, a clinical condition which is traditionally treated by surgical excision of affected tissues. Honey was found to be superior to orthodox treatment methods because it obviates the need for anesthesia and expensive surgical operation (Efem, 1993; Hejases *et al*., 1996). Honey has also been suggested to assist in the healing of leprous ulcers (Grange, 1990).

Honey has also been shown to be effective in treating a variety of ulcers (Keast-Butler, 1980; Mossel, 1980; Greenwood, 1993; Postmes *et al*., 1993). Dramatic improvement of gastric ulcers and gastritis after a treatment with honey has been observed (Salem, 1981). Laboratory trials found *Helicobacter pylori* infection (the probable causative agent of gastritis and duodenal ulcers) to be sensitive to a 20% solution of manuka honey (Al Somal *et al*., 1994).

Pure natural honey, applied every two-three days, promotes healing of decubitus ulcers (Blomfield, 1973) and ulcers in leprosy (Grange, 1990). Similarly, sickle-cell leg ulcers healed when treated with Eusol (a topical anti-microbial) combined with 12% honey (Ankra-Badu, 1992). From the many reports of clinical use, honey appears to successfully control septic wounds that commonly show a poor response to conventional therapy.

Honey has also been successfully used in the treatment of burns (Subrahmanyam, 1991; 1993). The beneficial effect of honey in the treatment wounds and burns is not restricted to its antibacterial action. It can also provide a viscous barrier preventing infection and fluid loss of the wound, can absorb oedema fluid and can act as an anti-inflammatory by relieving pain (Subrahmanyam, 1991). Some of these attributes of honey may help explain the evidence provided by Subrahmanyam (1993) for the successful skin grafts that were stored in honey for up to twelve weeks.

Abundant anecdotal evidence of the value of honey as a simple, convenient and effective topical remedy for infected, non-healing, skin wounds can be found in the literature (Armon, 1980; Bose, 1982; Green, 1988; Somerfield, 1991). During World War II, scarcities of medical supplies led to honey and lard being applied by soldiers to burns and wounds (Majno, 1975). In an animal model, honey applied topically to the open wounds of white mice healed significantly faster than the wounds of control animals (Bergman *et al*., 1983). Another study found that in 40 patients with wounds of various origins honey provided healing in 88% of cases (Ndayisaba *et al.*, 1992).

When honey was applied to open wounds from radical vulvectomy less bacterial colonisation and faster wound healing were observed (Cavanagh *et al*., 1970). A similar study found that honey can also be used to cure deeply infected abdominal wounds after Caesarean Section (Phuapradit, 1992). In a study of 59 patients with wounds that had failed to heal in response to conventional treatment, remarkable improvement was observed in 58 cases after topical application of honey. Wounds that were sterile at the outset remained sterile until healed, while infected wounds became sterile within one week of applying honey (Efem, 1988). Similarly, another study found that infected surgical wounds and bedsores became bacteriologically sterile within three days of honey application.

The usefulness of honey in treating wounds is not limited only to its antibacterial action. Honey promotes rapid growth of healthy granulation tissue (Armon, 1980; Efem, 1988; Hamdy *et al*., 1988). Honey also acts as an anti-inflammatory to relieve pain (Mladenov and Mladenova, 1985) and has a deodorising effect (Efem, 1988).

Honey has also been shown to be effective in other medicinal applications. Honey has proved effective in the treatment of infantile gastroenteritis (Haffejee and Moosa, 1985), shortening the duration of diarrhoea in patients. Independent researchers, Ali *et al.* (1991) and Al Somal *et al.*(1994) demonstrated that honey effectively inhibited *Helicobacter pylori*, the causative organism of acute gastritis and relapse of duodenal ulcer. Honey, because of its antibacterial action, anti-tussive and expectorant properties, is also included in the formulation of cough remedies and gargles treating laryngitis (Mladenov and Mladenova, 1985; Ali, 1989). However, in most of the reports, the type of honey used is not specified. Zumla and Lulat (1989) briefly reviewed the use of honey for therapeutic purposes and from the widespread reports of the clinical use of honey, they concluded that it was time for honey to receive due recognition by the medical world. Additionally, there is a worldwide upsurge of interest in therapies and remedies that are of natural origin rather than chemically synthesised agents. This, combined with the rapidly growing occurrence of multi-antibiotic resistant strains of bacteria, creates a need for alternative remedies.

Honey intended for medicinal use should be sterile and free of residues. Spores of *Clostridium botulinum* types A and B have been isolated from honey and have been implicated in the development of infant botulism (Midura *et al*., 1979). Although botulism contracted from infected wounds is not common (Merson and Dowell, 1973), there is a definite risk of introducing *Clostridium botulinum* into wounds if untreated honey is used as a dressing (Molan and Allen, 1996).

Honeys have been shown to be active against a diverse range of microorganisms, and reports of the inhibitory effect of honey on specific microorganisms are numerous. Molan (1992a) collated a comprehensive list in his review on the antibacterial activity of honey. Honey has been shown to be effective against Gram positive and Gram negative organisms, aerobic and anaerobic bacteria, as well as inhibiting spore germination of *Bacillus cereus* (El-Sukhon *et al.*, 1994). The sensitivity of different bacterial species and strains to honey is extremely variable.

Much research effort has centred on establishing the properties of honey to which its antibacterial activity may be attributed. Such factors as osmolarity, acidity, hydrogen peroxide content and chemical components of honey have all been considered to contribute to the inhibition of bacterial growth.

In the ancient use of honey as a medicine there was no knowledge of it having antibacterial properties, it was just known to work. Now it is known that festering wounds are the result of infection by microorganisms, and honey is used on the basis that it is an antimicrobial substance.

Honey is a highly saturated sugar solution which could suggest that this characteristic of honey would convey an antimicrobial effect. The high concentration of sugars leaves very little available water for the growth of microorganisms. However, the osmolarity of honey may not be the major factor. Dustmann (1978) has reported bacterial inhibition by very dilute solutions of honey (concentrations as low as 1.5%) and extremely low microbial inhibition has been observed using artificial honey with a sugar concentration similar to wild honey.

Honey is a supersaturated hyperosmotic solution of carbohydrates with a moisture content of 12-14% (Ruegg and Blanc, 1981). When microorganisms enter a hypertonic medium, the osmotic pressure differential is so great that organisms loose water (Burgett, 1990). Sustained dehydration will eventually kill any microorganism. Mean water activity values for honey have been reported as 0.562 (Ruegg and Blanc, 1981) and 0.5-0.6 (Troller and Christian, 1978). The growth of most bacteria and fungi is confined to the water activity range above 0.90 (Troller and Christian, 1978). Even osmotolerant microorganisms (halophilic bacteria and xerophilic fungi) require minimum water activities in the range 0.61-0.75 (Adams and Moss, 1995) for growth. Thus, the water activity of ripened honey is too low to support the growth of any microbial species (Molan, 1992b).

The antibacterial activity of honey involves more than the osmotic removal of water from micro-organisms and most of the antimicrobial tests used in different studies are carried out at honey concentrations where sugars are not osmotically active. In a study where honey samples were dialysed to remove sugar, *Staphylococcus aureus* was completely inhibited by 1.5 honey dilutions (Dustman, 1978). Similarly, greater microbial inhibition has been observed with natural honeys as compared with 'artificial honeys' (a solution of sugar of the same proportion typically in honey). In one study, 15 bacterial species were substantially (or completely) inhibited by 17% honey in agar, but were not inhibited by 'artificial honey' at the same concentration (Dold *et al*., 1937). *Staphylococcus aureus*, an organism noted for its exceptionally high tolerance of low water activity, is one of the bacterial species most susceptible to the antibacterial activity of honey (Molan, 1992a). Clearly, the antibacterial activity of honey can be ascribed to more than just the high sugar content of honey.

The acidity of honey has also been said to explain the antibacterial activity of honey. Characteristically honey is an acidic medium. Full strength honey pH ranges from 3.2 to 4.5 and is generally considered to have an average of 3.9 (White, 1975). Such an acidity level would be inhibitory to the growth of most bacterial species. Most microorganisms grow optimally at neutral (6.5-7.5) pH (Banwart, 1989; Adams and Moss, 1995). In general, bacteria grow optimally in the pH range 6.0–8.0, yeasts 4.5–6.0 and filamentous fungi 3.5-4.0 (Banwart, 1989). Thus, the degree of acidity in full strength honey would inhibit growth of most microbial species. Honey acidity is due primarily to the content of organic acids, predominantly gluconic (White, 1975). Gluconic acid in honey is generally thought to be produced by the enzymic action of glucose oxidase on sugar dextrose (White, 1992). However, some of the gluconic acid present in honey may be produced by bacteria of the genus Gluconobacter, which are occasionally isolated from ripening nectar (Ruiz-Argueso and Rodriguez-Navarro, 1973). Evidence from studies undertaken with neutralised honey has shown that antibacterial activity was retained (Wooton *et al*., 1978; Radwan *et al*., 1984). Also, under experimental conditions, it is likely that diluted honey would be neutralised by the buffering capacity of the bacterial growth medium and yet antibacterial activity is still observed. There appears to be no correlation between antibacterial activity and the acidity of the honey.

The major acid present in honey is gluconic acid (Stinson *et al.*, 1960) produced by glucose oxidase. Another compound produced by this reaction is hydrogen peroxide. Hydrogen peroxide is known to have antimicrobial properties and much evidence exists to suggest that it is this compound which confers antimicrobial activity to honey (Adcock, 1962; White *et al.*, 1963; Molan, 1992a). Hydrogen peroxide can be removed efficiently by the addition of catalase to the honey prior to testing for antibacterial activity (White *et al.*, 1963). There are, however, many reports of honeys retaining

partial or complete antibacterial potency following the addition of catalase (Molan and Russell, 1988; Molan, 1992a).

The presence in honey of non-osmotic antibacterial activity, termed “inhibine”, was first reported by Dold *et al*. in 1937. Inhibine was later identified as hydrogen peroxide produced enzymatically in honey (White *et al*., 1962; White, 1966). The enzyme glucose oxidase, secreted from the hypopharyngeal gland of the bee into nectar, assists in the formation of honey from nectar (Molan, 1996).

Gluconic acid serves to preserve ripening honey (Molan, 1996). Hydrogen peroxide has been recognised as an effective antibiotic for more than a century (Turner, 1983). Indeed, peroxide was a major antibacterial component of some early penicillin drugs, such as Notatin (Burgett, 1990). Hydrogen peroxide concentrations in excess of 0.0002% have been shown to prevent bacterial growth (White, 1966).

Researchers have found a direct correlation between anti-microbial activity and the non-peroxide activity of honey. Several researchers have proposed that the antibacterial activity exhibited by some honeys is due to a specific compound or group of compounds in the particular honey. In a study with honey that had been analytically shown to have no hydrogen peroxide and which took into account the osmolarity and acidity of the honey, Radwan *et al.* (1984) found honeys to retain antimicrobial activity against some bacterial and fungal species. They concluded that the inhibitory activity in honey was due to specific compounds. Roth *et al.* (1986) tested both catalase-treated honey and untreated honey, finding honeys retained the same antibacterial activity and so concluded that activity was not due to hydrogen peroxide.

The term ‘non-peroxide activity’ is used to describe any antimicrobial activity which does not arise from osmolarity, acidity or accumulation of hydrogen peroxide. The existence of non-peroxide antibacterial substances in honey is a controversial topic. Some authors argue that they account for an insignificant proportion (if any) of activity (Dustman, 1978; Morse, 1986) while others argue that they account for all of the activity beyond that due to acidity and high osmolarity (Mohrig and Messner, 1968; Radwan *et al*., 1984). Generally, it is accepted that both types of activity occur, to different degrees, in different honeys (Molan, 1992a). While evidence for the existence of non-peroxide antibacterial factors is primarily that the peroxide generating system does not account for all observed non-osmotic antibacterial activity, there have been some studies reporting the isolation of antibacterial substances from honey that are not hydrogen peroxide (Molan, 1992a).

The existence of non-peroxide antibacterial factors in honey is indicated by findings that the antibacterial activity does not correlate completely with the rate of accumulation of hydrogen peroxide in honey samples (Adcock, 1962; White and Subers, 1963; Dustman, 1978; Bogdanov, 1984). In one study, honeys producing significant quantities of hydrogen peroxide (when diluted) were not found to be antibacterial, while others that did not produce significant quantities of hydrogen peroxide were found to be antibacterial (Roth *et al*., 1986).

The most conclusive evidence for the existence of non-peroxide antibacterial factors in honey is that antibacterial activity persists in honeys treated with either catalase or peroxidase to remove hydrogen peroxide (Adcock, 1962; Bogdanov, 1984; Molan and Russell, 1988; Allen *et al*., 1991). New Zealand Manuka honey has a particularly high level of this type of activity (Molan, 1992a). The exact nature of honey’s non-peroxide antibacterial activity and the compounds responsible are not known.

In a large survey of New Zealand honeys, Molan and Russell (1988) found a correlation between high levels of antibacterial activity and non-peroxide content. Allen *et al.* (1991) suggested that the variation in activity of New Zealand honeys might be attributable to the floral source. Honey from Manuka (*Leptospermum scoparium*) demonstrated high antibacterial activity and this was shown to be due to a non-peroxide component.

There are reports of identification of some of the components of honey which are antibacterially active (Bogdanov, 1984; Toth *et al*., 1987; Russell *et al*., 1990). The identified compounds are different between the studies suggesting a possible floral derivation according to the honey type investigated. However, more research into this aspect is required.

In all the medical reports on the antibacterial and healing properties of honey, there has been little information given about the specific type or selection of honey used. The large survey conducted on honeys from different floral sources in NZ addressed this aspect and revealed some marked variation in the potency of antibacterial activity between honeys (Allen *et al*., 1991). From this finding, these workers stressed the importance of any particular honey being assayed for its antibacterial activity before consideration for therapeutic use.

To date, the only research on the antibacterial activity of Australian honeys is a small study by Wootton *et al.* (1978), which found that five of seven different floral sourced honeys did possess antibacterial activity. This activity was heat labile which suggests that the antibacterial activity present was due to hydrogen peroxide.

Considering the findings reviewed above there is sufficient evidence to suggest that honey may be the treatment of choice in wounds of all kinds. However, proof of efficiency in randomised scientific trials is lacking. More work in this area is clearly justified. However, few institutions are willing to conduct and finance such trials.

The current investigation was undertaken to establish the antimicrobial activity of a large number of Australian honeys from a wide range of floral sources and diverse geographical locations.

Beekeeper Guidelines

The development of guidelines for honey producers and processors is based on specifying the required handling parameters and conditions. Capilano Honey Limited, as the major honey processor in Australia, have been instrumental in sourcing "active" honeys for this project and in developing a medicinal honey product. For these reasons, it was decided that the guidelines would be best incorporated into the Capilano "Honey Manual". This manual meets Good Manufacturing Practice (GMP) requirements for the entire honey processing system (including the individual beekeeper) as well as Therapeutic Good Administration (TGA) requirements for those honeys which are antimicrobially active.

The specifications for antimicrobially active honeys have been largely based on knowledge obtained from research into the factors which may affect the activity of honeys. The considerations and procedures for apiarists collecting "active" or "medicinal" honey are very similar to those for apiarists collecting honey for food. Factors discussed and specified include the floral source of the honey flow, the importance of healthy beekeeping practices (especially the non-use of antibiotics within the hive), the extraction of honey without unnecessary heat treatment and the conditions for honey storage. Laboratory testing of any honey produced from "active" areas is essential to establish that the honey is antimicrobially "active". There is no prescribed method for the removal of this jelly-like (thixotropic) honey from the combs. The use of heat is to be avoided, as this may compromise the antimicrobial activity of the honey.

These guidelines outline recommended handling and processing procedures. Treatments which may compromise the antimicrobial activity of these honeys have been described. Any heat treatment of active honey should be avoided, if possible.

Characterisation of the “active” factor

Screening of honeys for antimicrobial activity

The antibacterial activity of honey was first reported in 1892 (Dustman, 1978). Research was reported next in 1919, however, extensive study did not begin until the work of Dold *et al*. in 1937. Intensive research has continued until the present day and involves the use of numerous microbiological techniques.

The most common technique used to study the potency of antibacterial activity in honey is the agar diffusion assay. A small quantity of honey is applied to holes bored into a nutrient agar plate pre-inoculated with the test culture. During incubation, the honey diffuses into the agar from its point of application. Where the concentration of honey in the agar is sufficient to inhibit growth of the culture, no colonies develop and a clear zone is observed around the point of application of the honey. The size of the clear zone is a measure of the potency of the honey. The major advantage of this method is that it allows comparisons of different honey types for the potency of their action against one or more species of bacteria. However, the method is limited because honey is diluted as it diffuses into the agar.

Turbidity tests and streak plates represent another technique to evaluate antibacterial activity. These systems involve incorporating honey, at different concentrations, into the nutrient agar or nutrient broth in which the culture is grown. Increased turbidity (as compared to an uninoculated control) is regarded as evidence of bacterial growth, and static turbidity as evidence of non-multiplication of the original inocula.

Several researchers (Roth, 1986; Smith, 1969; James, 1972) have also utilised the sensitivity disc method to assay the antibacterial activity of honey. This method involves saturating sterile filter paper discs with honey (undiluted or diluted) and applying them to the surface of agar plates seeded with the test organism. One suggested limitation of this method is that inhibitory compounds may absorb to the filter paper and thus inadequate concentrations diffuse into the agar, giving inaccurate results (James, 1972).

All the above methods are useful in investigating the activity spectrum of honey (*i.e.* determining which species of microorganisms are sensitive to the action of honey) but none of the methods can show whether the action of honey is bactericidal or bacteriostatic only. If no colony development occurs during incubation this can be regarded as a bacteriostatic action only (demonstration of bactericidal activity requires subsequent culturing in fresh nutrient medium to see if test microbes survived exposure to the honey).

Materials and Methods

Honeys

Honey samples were collected from commercial apiarists and processors throughout Australia. The floral source of each honey was identified by the beekeeper supplying it and most samples were considered to be monofloral specimens. Identification was based on colour, aroma and flavour, as well as location and season of production. Honeys were not submitted for pollen analysis to validate their identity due to the high cost involved. A preliminary investigation (Davis, 1997) tested a total of 340 honeys, and suggested that only one region, season and floral source was responsible for the floral-active honeys found throughout Australia. In this study, honey samples supplied by apiarists and by Capilano Honey Limited were largely derived from *Leptospermum* trees (or honeys which had a character similar to *Leptospermum* honey). Honey samples were held in airtight glass or plastic containers in the absence of light to minimise any changes in honey composition. All samples were frozen and stored at $^{-}20^{o}$C. For assaying, the honeys were allowed to come to room temperature prior to sampling. Care was taken to avoid any obvious pieces of comb wax and the surface portion of the honey which may have had greater exposure to oxygen and light.

Artificial honey

An artificial honey mixture was prepared by adding gluconic acid lactone to sterile deionised water until pH 3.8 was achieved following lactone hydrolysis. To 17.5mL of the gluconic acid solution, 40g fructose, 36.2g glucose and 2.8g sucrose were added and heated to 50^{o}C to dissolve the sugars. This typical honey solution was used in the standard assay procedure to determine the effect of sugar concentration or acidity on antimicrobial activity.

The test bacterial strain

Staphylococcus aureus (ATCC 25923) was used for screening all honeys for antimicrobial activity. This bacterial strain was chosen as it is the one most commonly used by other researchers for assessing the antimicrobial activity of honeys (Molan *et al*., 1988). Additionally, this microorganism is known to be tolerant of the high concentration of sugars and the acidity of honey while being sensitive to hydrogen peroxide (Dustmann, 1979) and the chemical inhibitory action of honey (Molan and Russell, 1988).

The test bacterial strain

Staphylococcus aureus ATCC 25923 is used experimentally for testing all honey samples and honey fractions for antimicrobial activity. This bacterial strain is the most commonly chosen by researchers assaying antimicrobial activity in honey (Molan *et al*., 1988) and allows for greater comparison with previous research. Additionally, *Staphylococcus aureus* ATCC 25923 is tolerant of the high sugar concentration and acidity of honey while being sensitive to the antimicrobial action of hydrogen peroxide (Dustman, 1978) and the non-peroxide inhibitory action of honey (Molan and Russell, 1988). Variation of antibacterial activity between Australian honeys from different floral sources and within a single floral source is large (Davis, 1997). This phenomenon has also been observed in the antibacterial activity of New Zealand honeys (Allen *et al*., 1991). Indeed such variation in activity between and within different honey samples is common to almost all studies in which more than one honey type has been investigated (Molan, 1992b).

Other bacterial strains

Other bacterial strains used in the diffusion assay were: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Streptococcus*, *Bacillus circulans*, *Bacillus cereus*, *Yersinia enterocolitica* (ATCC 9610), *Listeria monocytogenes* (NCTC 11994) and *Micrococcus luteus* (ATCC 4698). These bacterial strains were selected to give an indication of the antimicrobial activity of honeys against both pathogenic and non-pathogenic bacteria. Bacterial isolates were also collected from the DPI Mastitis Collection (Animal Research Institute) to assess the efficacy of *Leptospermum* honey against these bacteria. A range of antibiotic-resistant organisms (*S.aureus* and *Pseudomonas spp*.) were also assessed in the Department of Microbiology at the Royal Brisbane Hospital.

Agar plate preparation

An overnight culture of *Staphylococcus aureus* was grown at 37^{o}C for 18 h in 10mL nutrient broth (Oxoid No. 2). One mL of the overnight culture was inoculated into 100mL nutrient broth and incubated with shaking (4000 rev/min) at 30^{o}C for 3 h to attain a fully logarithmic phase culture. A 1.8mL aliquot of log phase culture was added to 46mL of nutrient agar (Oxoid No. 2) nutrient broth plus 1.5% agar, Bacteriological grade, BBL), maintained to 45^{o}C. The final bacteriological concentration was 10^{3}-10^{4} cfu/mL. The seeded agar was immediately poured into large plastic petri dishes (150 x 19 mm, Nunc). Once solidified, wells were cut in the agar with an 8 mm cork borer according to a standard template. Agar plugs were removed under sterile conditions.

Assay procedure

For assaying, honeys were diluted to 25% (w/v) in either sterile deionised water or catalase solution (Sigma, EC 1.11.1.6, bovine liver: 2800 units/mg) at a concentration of 8000 units/mL. At this dilution, the antibacterial activity of honey is linearly proportional to the diameter of the zone of inhibition (Cooper, 1963; Allen *et al*., 1991). A test sample of 80µL of diluted honey was used to completely fill the well in the agar plate. Honeys were always tested in triplicate, dispensed randomly into wells with each plate including a negative control (sterile deionised water) and a positive control

(6% phenol). The positive control gave a zone of inhibition which was used to correlate for day-to-day variations. The plates were incubated overnight at 37°C. Antibacterial activity was indicated by a zone of no growth surrounding the well and was determined by measuring the diameter of the clear zone (mm). All measurements were recorded without reference to the sample identity.

Results and Discussion

Agar well diffusion assay

Of all the methods available for determining antimicrobial activity, the agar well diffusion method was selected for its convenience, low cost and rapidity. It allows reliable and reproducible comparison between large numbers of samples. Additionally, this assay is the most appropriate for testing topical antimicrobial agents as it takes into account the diffusibility of the agent into infected areas with little or no blood supply (Heggers *et al*., 1987). The assay is an end-point test and therefore results obtained are qualitative or, at best, semi-quantitative when a high degree of standardisation is employed. It is not a highly sensitive assay (James *et al*., 1972) as the honey sample is further diluted by diffusion into the agar. Diffusion is slow and hence the colonies on the outer limit can grow prior to the inhibitory substance reaching them. The size of the inhibitory zone achieved is dependent upon rates of diffusion and cell growth (Barry, 1986). This infers that the extent of the zone of inhibition may not necessarily be directly proportional to the activity of the honey sample. In an agar diffusion assay, Cooper (1963) established that the logarithm of the concentration of an antibacterial substance is proportional to the square of the extent of the zone of inhibition. For the research work in this study, the agar well diffusion method was used for the qualitative comparison of the antimicrobial activities of different honeys.

Antibacterial activity of honeys

In this three-year study, the honey samples supplied were predominantly obtained from Capilano, and they were largely selected for their typical *Leptospermum* characteristics. Consequently, a far greater proportion of samples returned a positive result for antimicrobial activity than in the previous study (Davis, 1997). Invariably, the honey samples which tested positive for antimicrobial activity were *Leptospermum*-derived. This study has also confirmed that one particular area in Northern New South Wales (in the Broadwater National Park) has repeatedly (for at least 4 seasons) produced honey with floral-derived antimicrobial activity. At least two further distinct regions (in Queensland) have recently been identified as producing active *Leptospermum* honey for at least one season.

In this three-year study, the vast majority of "active" honeys were floral-active honeys (*i.e. Leptospermum*-derived). In the 1998 year, a total of 1006 honey samples were screened. Of these, 76 were floral active and 26 were peroxide active (7.5% and 2.6%, respectively). In the 1999 season, 238 of the 929 samples (26%) were floral active while only one sample was peroxide active. In the third year (2000), 359 of the 708 samples (51%) were floral active with no peroxide active honeys tested. This suggests that the honey supply (mainly from Capilano) was being more accurately targeted to the *Leptospermum* honeys as the project progressed. The number of active honeys tested as the project progressed may not be entirely accurate, as samples may have been submitted for reanalysis without being accounted for. This may have included testing of samples after mixing and processing.

In a previous study (Davis, 1997) where 340 honeys were screened, 31.5% demonstrated antibacterial activity against *Staphylococcus aureus* (ATCC 25923). Where reasonable numbers of samples were obtained from the same monofloral source, several specific floral varietals occasionally produced honeys with antibacterial activity. These floral sources include Bimble box, Yapunyah, Grey Ironbark, Jelly bush, Salvation Jane and Crow ash. The former three species are from the Eucalyptus genus. In a study of seven Australian honeys by Wootton *et al*. (1978), Stringy Bark and Yapunyah were also shown to have high antibacterial activity. Jelly bush and Salvation Jane come from the genus *Leptospermum* and *Echium* respectively and in a study of New Zealand honeys (Allen *et al*., 1991), it was also found that species from these two genera were more likely to produce honeys with floral-derived antibacterial activity.

The variation of antibacterial activity between honeys from different floral sources and within a single source is large. This phenomenon was also observed in a similar survey of antibacterial activity of New Zealand honeys (Allen *et al*., 1991) and in other research (Molan *et al*., 1988). In a recent review of antibacterial activity of honey, Molan (1992a) reports that such variation in activity between different honeys is common to almost all studies in which more than one type of honey has been investigated.

Of the many factors discussed in attempts to explain the large activity variations seen, some can be ruled out. Age of the honey (storage time) and processing/handling factors were shown to have no effect on the antibacterial activity present in a large number of New Zealand honeys (Allen *et al*., 1991). Sensitivity of the assay system may influence the antimicrobial activity. For the results presented in the current work, the agar well diffusion assay may not have been able to detect very low levels of antibacterial activity which may have been present in some of the honey samples.

It is suggested that honeys from certain floral sources are more likely than others to have antibacterial activity. However, Molan (1992a) concluded that there is not enough evidence for such definite conclusions to be justified. Not all honeys derived from a particular floral source have antibacterial activity. Many honey samples demonstrated no activity. In this three-year study, large sample populations were screened and the jelly bush honey was shown to repeatedly produce positive results.

The honeys screened in a previous study (Davis, 1997) were sampled from a very wide range of apiarists making it difficult to draw any conclusions concerning a relationship between the geographical location of the floral source of the honey or the season of production and antimicrobial activity. This investigation has been largely focussed on honeys with the particular *Leptospermum* characteristics observed in the earlier study (Davis, 1997).

The antimicrobial activity of honey can be attributed in part to the presence of hydrogen peroxide in the honey (White *et al*., 1963). Hydrogen peroxide can be destroyed by catalase and it is known that different plant species contain different catalase activities (Schepartz, 1966; Schepartz and Subers, 1966). This would have an influence on the level of hydrogen peroxide present in the honey and hence the antimicrobial activity. Therefore, it is reasonable to expect a correlation between floral source and antibacterial activity and to believe this to account for the variation seen between honeys from different floral sources. However, such a correlation does not explain the variation observed within a floral source. This variability is not unique to the current work, but was also commented on by Allen *et al*. (1991) who observed a very marked variation in the level of hydrogen peroxide activity within a single floral source. Interestingly, the active honeys sampled for this three-year study were predominantly active due to floral-derived factors. This may be because the collection was focussed on *Leptospermum* type honeys.

Molan *et al*. (1988) decided there was a relationship between antibacterial activity and the floral source of the honey, with Manuka honey showing significantly higher activity than other floral sources. Allen *et al*. (1991) concluded that not all samples of Manuka honey can be relied on to have activity. This again indicates the wide variation within a specific floral source.

A likely rationalisation for the variation in activity, both between and within floral sources, arises from the nature of honey production. Bees are able to take nectar from whatever source is available to them at the time and hence it is improbable that straight line or strictly monofloral honeys would ever be obtained. The validity of identification of the honey as occurred within this research and that of others, is open to question. It is often based on experience and “best guess” according to season and location of the hives. Pollen analysis would provide definitive identification of the honeys but the analysis is expensive and rarely done.

The agar well diffusion assay as used in this work, allows relative comparisons of activity between honeys. For the honeys tested, a sample from Jelly Bush had an activity equivalent to that reported by other researchers (Molan and Russell, 1988; Molan *et al*., 1988; Allen *et al*., 1991). Direct comparison of results from different workers is often difficult due to differences in assay procedures

involving different honey concentrations and test microorganisms used. Discussions are currently underway to standardise the agar well assay system between New Zealand and Australian researchers so that more reliable comparisons can be made.

Non-hydrogen peroxide activity

Catalase is able to degrade hydrogen peroxide and therefore can be used to remove any antimicrobial activity which is attributable to hydrogen peroxide from honey. Molan and Russell (1988) demonstrated the efficacy of a weaker enzyme solution than the one used for this research. All honeys that exhibited antimicrobial activity were tested following the addition of catalase solution. As the reference bacterial strain used for screening is resistant to osmotic and acidic effects of honey, it is likely that any retained activity is due to a compound of floral derivation. To show that non-peroxide antimicrobial activity was not due to the presence of traditional chemical antibiotics, samples of honeys exhibiting non-peroxide activity were submitted for mass spectrophotometric analysis. No sample returned a positive result for the range of antibiotics tested.

Allen *et al*. (1991) tested honeys of New Zealand floral origin and found that only two floral varietals (*Leptospermum scoparium* and *Echium vulgare*) produced honey with non-peroxide activity in a significant proportion of the samples. Our initial research with Australian honeys indicates that a *Leptospermum*-derived honey (probably *Leptospermum polygalifolium*) had the most consistent and significant non-peroxide activity. No other reports have described the activity of Australian honeys following removal of hydrogen peroxide.

For the honeys having non-peroxide activity, the inhibitory effect on *Staphylococcus aureus* (ATCC 25923) remains similar to the total antibacterial activity present. This suggests that all the activity present in the honey is due to a non-peroxide component, presumably of floral derivation. This is similar to the findings of Molan and Russell (1988) in their study of New Zealand honeys where they concluded that in the honeys with high antibacterial activity, a large part of the activity was due to a factor other than hydrogen peroxide. In contrast, Dustman (1979) reported non-peroxide activity in honey but concluded that it was only a minor proportion of the total antimicrobial activity present.

The non-peroxide activity of some jelly bush honeys is equivalent to the “active” Manuka honeys described by Molan *et al*. (1988). Direct comparison of the activities of the honeys is appropriate since we were able test the “active” Manuka honey under our assay conditions (thanks to the kind sample donation and collaboration of Professor Peter Molan). The “active” Manuka honey and the Australian jelly bush honey both repeatedly exhibited an inhibition zone of about 14 mm when tested after the addition of catalase. Molan has stated that the activity of the Manuka honey is as high as the most “active” honey recorded elsewhere. Therefore, by direct implication, so is the activity of the jelly bush honey.

Relative activities between honeys

Jelly bush honey is a dark coloured, strong tasting honey and it is interesting to note that several studies have commented on similar characteristics for honeys found to have high antibacterial activity. Manuka is a dark coloured honey, as is that from the central European conifer forests and the sweet chestnut. Also, dark coloured Canadian honeys were associated with high activity (Molan, 1992). The addition of catalase to Australian floral sourced honeys with high antibacterial activity (zone of inhibition >11 mm) resulted in the total abolition of activity. The results in this work also demonstrated honeys with relatively low antibacterial activity that was all attributable to a non-peroxide component of the honey.

Stability of the activity

Exposure of the most active jelly bush honey to 60^{o}C, 80^{o}C and 100^{o}C for 18 h resulted in no reduction of antibacterial activity when the honey was tested with or without catalase. Similarly, exposure of the honey to UV radiation for 18 h caused no loss of activity. These findings further indicate that the activity is due to a non-peroxide component in the honey, as hydrogen peroxide would be inactivated by such treatments (Molan and Russell, 1988).

Microorganism spectrum of the antibacterial activity

Honeys were assayed against a range of microorganisms to determine whether they are active against the specific bacteria. The activity of honeys against a much wider range of microorganisms, including clinically significant microbes and pathogens, as well as non-pathogenic organisms especially those sourced from food processing/hygiene environments, was undertaken. For screening purposes, the standard strain *Staphylococcus aureus* (ATCC 25923) was used at all times for reasons mentioned previously. However, if honey is considered to have potential for therapeutic use in a clinical situation it is important to know which, if any, other bacterial species are susceptible to the action of honey. The antibacterial activity of honeys against those microorganisms which exhibit resistance to conventional pharmaceutical drugs shows considerable promise.

An investigation of the anti-microbial efficacy of jelly bush honeys against a range of food spoilage organisms was undertaken by Brenda Mossel, a PhD candidate from the University of Queensland, Gatton, and these results will be presented in her dissertation. The efficacy of *Leptospermum* honeys against the reference strain S.*aureus* (ATCC 25923), food spoilage microorganisms and food pathogens was investigated. Food spoilage microorganisms included thermophilic spore-forming bacteria *Bacillus stearothermophilus*, yeast (*Zygosaccharomyces bailii* and *Z. rouxii*), *Pseudomonas (P.fluorescens, P.putida* and *P.cepacia)* and *Lactobaccillus (L.plantarum).* Food Pathogens included *Bacillus cereus* (ATCC 49664), *Staphylococcus aureus* (ATCC 13625), *Pseudomonas aureginosa* (ATCC 15022), *Pseudomonas aureginosa* (ATCC 12066), *Vibrio parahaemyliticus, Salmonella* spp. and *Listeria monocytogenes*. All of the food pathogens assayed in this study except *Pseudomonas aureginosa* strains were inhibited by *Leptospermum* honeys.

In a study undertaken at the Royal Brisbane Hospital, 100 clinical isolates of antibiotic-resistant strains of *Pseudomonas aeruginosa* and a similar number of antibiotic-resistant *Staphylococcus aureus* strains were exposed to a range of concentrations of active jelly bush honey. The *Pseudomonas* cultures were all completely inhibited from growing by concentrations of honey greater than 4.5% while the *Staphylococcus aureus* cultures were all completely inhibited from growing by concentrations of honey greater than 7%. The efficacy of Australian jelly bush honeys against medical pathogens was also investigated by Shona Blair (a PhD candidate from the University of Sydney). Shona will present a range of work relating to the medical application of these honeys in her PhD dissertation. Much of Shona's research has focused on the mechanism of action of honey from a medical perspective, both through its antibacterial activity and its possible involvement in cytokine activation. She has investigated the stability and characteristics of the active agent and the mode of action of honey in wound healing. Both of these dissertations are currently in preparation, and the detailed outcomes of these studies will be available in the near future.

Chemical screening of the honey

A thorough chemical investigation of Australian honeys was largely undertaken by Brenda Mossel, a PhD candidate from the University of Queensland, Gatton. Her work has aimed to correlate antimicrobial activity with chemical components in unifloral honeys. This investigation initially involved fractionating ‘active’ Jelly bush (*Leptospermum polygalifolium*) honey. These honeys were identified in a previous study (Davis, 1997) as the only unifloral Australian honey to repeatedly display significant antimicrobial activity against the test reference strain of *Staphylococcus aureus* ATCC 25923. The aim of this work was to identify the chemical components responsible for the non-peroxide floral-derived antimicrobial activity. To date, the factors responsible for this floral-derived anti-bacterial activity have remained elusive. The methods developed and the results of these largely unsuccessful fractionations of "active" honeys are detailed in Brenda's PhD report.

The second part of Brenda's research involved the chemical screening of a number of unifloral Australian honeys (including samples of active and non-active Jelly bush honeys) and New Zealand Manuka honey (both active and non-active samples) to see if the active honeys are chemically distinct. Numerous studies have been conducted attempting to isolate the factor or factors responsible for the antimicrobial activity in honey. However, attempts to correlate antimicrobial activity with the physical and chemical parameters of honey are limited. This research has attempted to identify

components that may be therapeutically important, particularly in moist wound management. Colour, moisture, ash, electrical conductivity, specific rotation, proline, HMF, invertase, diastase, carbohydrates (fructose, glucose, sucrose, maltose and turanose), granulation indices, pH, free acidity, lactone acidity and total acidity were determined for 138 monofloral Australian honeys from 15 common honey types. The results of this work give a thorough overview of the chemical composition of Australian honey. The last such investigation was undertaken over 20 years ago.

Honey samples from a honey variety acknowledged to display antimicrobial activity, such as Australian jelly bush (*Leptospermum polygalifolium*) and New Zealand Manuka (*Leptospermum scoparium*), can not be relied upon to invariably have antimicrobial activity. Similarly, antimicrobial activity is recorded in jelly bush and Manuka honeys from quite specific geographical localities. The aim of this study was to determine if there was any correlation between antimicrobial activity and the rheological and chemical parameters of monofloral Australian honey samples. While most of the chemical analyses did not correlate the chemical characteristics of floral active honey with the presence of anti-bacterial activity, two components did show some promise. Anisic acid was shown to associate strongly with non-peroxide antimicrobial activity. The reaction of hydrogen peroxide with anisic acid may create a peroxycarboxlic acid, which are powerful antimicrobial agents. This peroxide, formed from hydrogen peroxide and anisic acid (from the nectar of chemovars providing active honeys), is not destroyed by the added catalase and may be responsible for the non-peroxide antimicrobial activity observed in this study. Alternately, hydroxymethyfurfural (a Maillard reaction product) and proline (the principle amino acid in honey) were significantly higher in active honeys and associated strongly with non-peroxide antimicrobial activity. This suggests that active honeys may have been stored under conditions favourable for Maillard reaction product formation. In additon, the honey darkening observed in floral active honeys is one of the consequences of Maillard reactions. Maillard reaction products are potent antimicrobials. The level of non-peroxide antimicrobial activity in active jelly bush honey samples (*Leptospermum polygalifolium*) increases during warehousing, suggesting that some form of reaction product is responsible. Thus, a Maillard reaction product between glucose and an amino acid or peptide unique to the nectar of the chemovar or sub-species of *Leptospermum* producing active honey, may be responsible for the non-peroxide antimicrobial activity in *Leptospermum* honey. The detailed outcomes of these studies will be available when Brenda's PhD dissertation is completed.

Conclusion

The use of honey as a therapeutic agent dates to ancient times. More recently, there has been growing interest in this 'natural' remedy, which has lead to legitimate scientific investigations. Research in New Zealand has shown that Manuka honey has very special healing properties. This honey has been described to contain "the best natural antibiotic in the World". There is much evidence to suggest that the antibacterial activity of honey is due to more than the sugars present therein. Two major sources have been shown to be responsible for the anti-microbial activity - hydrogen peroxide (from the bee) and uncharacterised chemical substances (from the floral source). Using honeys sourced from around Australia and screened against several reference microorganisms, the anti-microbial potency of one particular Australian honey was shown to be comparable to NZ Manuka honey. Initial chemical comparison has confirmed that the NZ Manuka and the "active" Australian jelly bush honey are very similar. This is not unexpected since both of these remarkable honeys are derived from *Leptospermum* trees.

Previous research at the Centre for Food Technology has identified Australian honeys with anti-microbial activity . This research project will endeavour to give credibility to the use of honey as a treatment for moist wound injuries. The mode of action of the various honeys, particularly those derived from *Leptospermum* species (ie bacteriostatic or bactericidal) and the susceptibility of various food and medical pathogens to "active" honey varieties was investigated. Standard microbiological techniques were employed and the honeys were tested against a variety of potential pathogens. While the initially studies used less virulent microorganisms, later tests were performed with the antibiotic-resistant microorganisms (*i.e.* Golden *Staph* (MRSA) and Vancomycin-Resistant Enterococcus (VRE)) which are major problems in hospitals throughout the World.

This project was undertaken to add value to the existing honey resource by developing products with therapeutic benefit (*i.e.* for the treatment and management of moist wounds such as burns and ulcers). The production of such valuable honeys requires the honey to be collected and processed under prescribed conditions. This involves the identification of the appropriate floral sources, the development of procedures for harvesting and handling, the evaluation of the "active" agent(s), and the registration of honey as a therapeutic agent.

In an Australia-wide context, the honey and pollen industries are estimated to be worth in the order of $A32 M (which does noes not include the value of incidental pollination of many agricultural crops). This project has significant potential to add value to the Australian Honey Industry. The value of honey sales to the New Zealand Honey Industry has increased significantly with the research and associated promotion of their native Manuka honey. The research undertaken in this project and extension of these results has promoted the use of honey for the treatment of bacterial infections associated with such injuries as burns and ulcers.

Honeys have been shown to be active against a diverse range of microorganisms and reports of the inhibitory effect of honey on specific microorganisms are numerous. Honey has been shown to be effective against both Gram positive and Gram negative organisms, aerobic and anaerobic bacteria, as well as inhibiting spore germination of *Bacillus cereus*. The sensitivity of different bacterial species and strains to honey is extremely variable. Honey has also recently been shown to have an inhibitory effect against antibiotic resistant strains (*e.g.* golden *Staph*), which are frequently responsible for post-operative wound infection in immunologically compromised patients.

Much research effort has centred on establishing the properties of honey to which its antibacterial activity may be attributed. Such factors as osmolarity, acidity, hydrogen peroxide content and chemical components of honey have all been considered to contribute to the inhibition of bacterial growth. Honey is a highly saturated sugar solution which could suggest that this characteristic of honey would convey an antimicrobial effect. The high concentration of sugars leaves very little available water for the growth of microorganisms. However, the osmolarity of honey does not appear to be a major factor. The acidity of honey has also been suggested to explain the antibacterial activity

of honey. Honey contains many organic acids, predominantly gluconic acid produced from glucose by glucose oxidase, and is characteristically acidic with pH 3.2 to 4.5. Although such an acidity level would be inhibitory to the growth of most bacterial species, there appears to be no correlation between antibacterial activity and the acidity of the honey. There is much evidence to suggest that the antibacterial activity of honey is due to more than the sugars present therein. Two major sources have been shown to be responsible for the anti-microbial activity - hydrogen peroxide (from the bee) and uncharacterised chemical substances (from the floral source). Hydrogen peroxide is known to have antimicrobial properties and can be removed efficiently by the addition of catalase to the honey prior to testing for antibacterial activity. The research undertaken in this project has been directed at understanding the correlation between antimicrobial activity and the non-peroxide activity of particular *Leptospermum*-derived honeys.

Research in the project has been focused on the specific antimicrobial potency of *Leptospermum* honey. Initial investigations tested the efficacy of these honeys against one particular bacteria (*Staphylococcus aureus*), while later studies assessed the effect of this honey on a range of food pathogens, animal pathogens (*e.g.* in mastitis) and human pathogens (*e.g.* golden *Staph*). Using honeys sourced from around Australia and screened against several reference microorganisms, the anti-microbial potency of one particular Australian honey (from *Leptospermum polygalifolium*) was shown to be comparable to NZ Manuka honey (from *L.scoparium*). The results of this screening of active honeys against pathogenic bacteria has supported the registration of honey by Capilano Honey Limited as a "Drug" with the Therapeutic Good Administration based on its antimicrobial activity.

This project was undertaken to add value to the existing honey resource by developing products with therapeutic benefit (*i.e.* for the treatment and management of moist wounds such as leg ulcers). Specific geographic region(s) in Australia which produce "active" honey have been identified, the potency of the *Leptospermum*-derived honeys against a range of bacteria has been defined, the particular chemical characteristics of these honeys have been examined, and the honey has been registered as a "Drug" with the Therapeutic Good Administration. Work is continuing to better define the specific agents responsible for the antimicrobial activity (which have been elusive to date) and to evaluate the opportunity for therapeutic benefit from honey beyond its antimicrobial activity (*i.e.* its direct wound healing benefit). Routine analysis of the antimicrobial activity of honey is now undertaken at the Centre for Food Technology using the agar well diffusion assay. This allows us to directly compare the results from different laboratories (*e.g*. New Zealand testing laboratories). Discussions are currently underway to better standardise the agar well assay system between New Zealand and Australian researchers so that more reliable comparisons can be made.

Findings from such further work would develop a far greater understanding of the nature of the antibacterial activity of our specific honeys and thereby illustrate the therapeutic potential. The improved understanding of honey as a natural antibacterial agent would increase the marketed value of the honey and hence improve revenue to Australian beekeepers.

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The Use of Australian Honey in Moist Wound Management

RIRDC Pub. No. 05/159

This RIRDC report investigates the correlation between antimicrobial activity and the non-peroxide activity of particular *Leptospermum*-derived honeys.

Initial investigations tested the efficacy of these honeys against one particular bacteria (*Staphylococcus aureus*), while later studies assessed the effect of this honey on a range of food pathogens, animal pathogens (e.g. in mastitis) and human pathogens (e.g. golden Staph).

Using honeys sourced from around Australia and screened against several reference microorganisms, the anti-microbial potency of one particular Australian honey (from *Leptospermum polygalifolium*) was shown to be comparable to NZ Manuka honey (from *L.scoparium*). The results of this screening of active honeys against pathogenic bacteria has supported the registration of honey by Capilano Honey Limited as a "Drug" with the Therapeutic Good Administration based on its antimicrobial activity.

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