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Eucalyptus marginata (Jarrah)

Research into Western Australian honeys

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Antimicrobial & antioxidant activity

Background

Evidence of “active” honey was discovered when a positive random test for antibiotic residue in honey samples from one of Western Australian (WA) packers proved to be false after honey samples were re-run using a more expensive test.

Khaira and Mee (2000) at the Department of Microbiology, The Queen Elizabeth II Medical Centre, University of Western Australia examined the minimum inhibitory concentrations (MIC) of various WA honey samples (from the August 1999 to January 2000 flowering season). Jarrah honey was a most active honey against microbes than all others tested. Its MIC for the most active Jarrah sample was 20% w/v (20 g honey in 100 mls water) for *Candida albicans*, 5% w/v for *Pseudomonas aeruginosa* and *Escherichia coli*, and 2.5% w/v for *Staphylococcus aureus*. *S. aureus* was the most sensitive microbe to Jarrah honey.

On the 26 February 2002, the then Department of Agriculture released a media statement about the results of research conducted by Rob Manning and Nola Mercer about WA honeys’ antimicrobial activity using an assay developed in New Zealand. The research showed that Western Australian honey had some of the highest activity levels in the world due to a naturally occurring enzyme in the honey. Upon dilution of honey, the enzyme glucose oxidase produces low concentrations of hydrogen peroxide which is the source of its antimicrobial activity. It is different to Manuka (*Leptospermum scoparium*) honey from New Zealand which is termed a ‘non-peroxide’ (Molan and Russell 1988) honey because it’s activity is derived from a chemical called methylglyoxal. The chemical, the “Unique Manuka Factor (UMF)” in Manuka is unique and so far it has only been discovered in honey from *Leptospermum* species (Allen *et al.* 1991; Anon 1998; Davis 2005) which includes a species found in Western Australia (Beeinformed 2008).

Since 2002, further research into WA honeys has been undertaken by researchers at the University of Sydney, Western Australian beekeepers and Wescobee Ltd. In 2009, Wescobee Ltd launched a new product that promoted a 100% honey product

based on its antimicrobial activity into a health-care market for the first time and with it won an export award into Japan in 2010 (Wescobee 2011).

The Jarrah honey crop has been significantly affected by adverse environment conditions, particularly by drought for many of the years since 2002.

This bulletin provides a history of the discovery of active honeys in WA and more recently antioxidant activity.

Introduction

Honey is a highly complex mixture of at least 200 phytochemicals whose composition is strictly dependent on floral and geographical origin (Beretta *et al.* (2005). The antimicrobial activity of honey is due to its osmotic effect, acidity and the presence of hydrogen peroxide (Molan 1992) which can vary markedly between samples (Molan *et al.* 1988; Garcia *et al.* 2000; Brady *et al.* 2004). The hydrogen peroxide activity can be measured, and is now important in grading the activity of the honey in qualitative terms for the pharmaceutical-medical market, specifically in wound care and for skin infections.

Hydrogen peroxide presence in honey is derived from the enzymatic activity of glucose oxidase. The source of glucose oxidase found in honey can originate from the honey bees' hypopharyngeal glands that are located within its head (Gauhe 1941, cited in Weston 2000) or from the nectar of the flower (Carter and Thornburg 2004).

Hydrogen peroxide is found in varying concentrations in honeys (Allen *et al.* 1991) and is produced optimally when honey is diluted to concentrations between 30 - 50% (v/v) (Bang *et al.* 2003). However, results from a number of surveys can show samples of honey with little or no antimicrobial activity (Allen *et al.* 1991; Brady *et al.* 2004).

Honeys with a high antimicrobial activity have achieved medical status as being important effective antiseptic alternatives when dressing wounds, burns and ulcers (Molan 2001).

Survey of honeys for antimicrobial activity

A survey of unpasteurized honey from commercial Western Australian apiarists was carried out on 342 honey samples from the south-west of WA from 1999 to 2004. The honey was tested against *Staphylococcus aureus* 'Golden Staph' in an agar well diffusion assay, with reference to phenol as a standard.

Honey assay for antimicrobial activity

The methodology used was similar to that used by the Honey Research Laboratory, University of Waikato, Hamilton, New Zealand, where the agar well diffusion method (Fig 1) used and adapted the punch plate assay for inhibitory substances described in Allen *et al.* (1991).

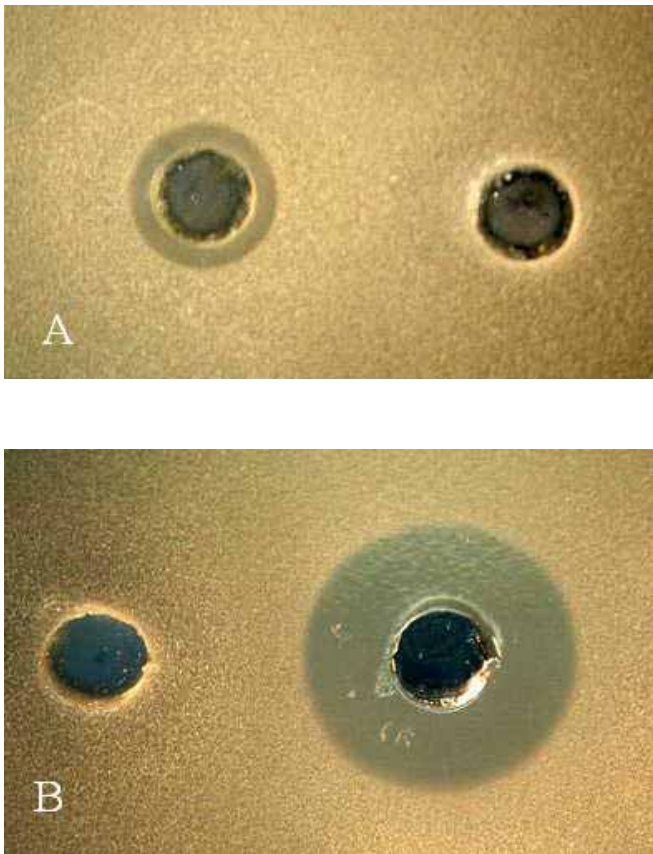


Fig 1. Agar well diffusion assay showing the inhibitory zone preventing the growth of *Staphylococcus aureus* (ATCC 9144). Some honeys antimicrobial activity is less (A: Top left) than Jarrah honey (B: Bottom right), which shows the large clear inhibitory zone around the well cut into the agar.

However, the *Staphylococcus aureus* isolate type used in Manning and Mercer (unpublished) differed, being a freeze-dried culture of isolate

ATCC 9144 obtained from the Centre for Food Technology, Department of Primary Industries, Queensland. It was reconstituted in Trypticase Soy broth (Merck 1.05459) according to the instructions supplied, and incubated at 37°C for 18 hours.

Other parts of the methodology that differed to Allen *et al.* (1991) were the nutrient agar brand (23g/l OXOID CM 3), catalase brand (Sigma C9322, 2800 units/mg) and the phenol standard (10% w/v solution) was analytical reagent grade (BDH).

A previously screened Manuka honey sample (M110) from the University of Waikato (New Zealand) was tested without any knowledge of its known activity and the WA test result was confirmed in NZ as being as accurate as NZ data. On this basis, regular testing of 'M110' was conducted to ascertain the robustness of the methodology over time during the survey.

Results

*Antimicrobial activity of honeys*¹

Clinically, the medical interest is in active honey. New Zealand's iconic 'medihoney', the honey from Manuka is sold at specific activity levels. Therefore only the active honey data is presented here. Of the 342 honey samples, 70 samples (20.5%) showed no detectable antimicrobial activity and the distribution of activity of the honeys surveyed varied greatly from 0 to 45% (w/v) phenol (Fig. 2).

Two endemic Western Australian honeys derived from Marri (*Corymbia calophylla*) and Jarrah (*Eucalyptus marginata*) trees were found to have consistently high antimicrobial activity of all honeys surveyed with measured average activity of 31.1% w/v phenol (29)² and 30.2% (w/v) phenol (62), respectively. The activity of the two endemic species was about 90% greater than the average activity of New Zealand's manuka honey (Fig. 3).

¹ The test values are expressed as % w/v phenol and can be expressed as % phenol equivalent because the antimicrobial activity test is a **phenol equivalence assay** where the activity of honey is referenced to a phenol standard. Many species had samples that were non-active and some species had few sample collected e.g. 1-3.

² The number following the phenol reading is the sample size.

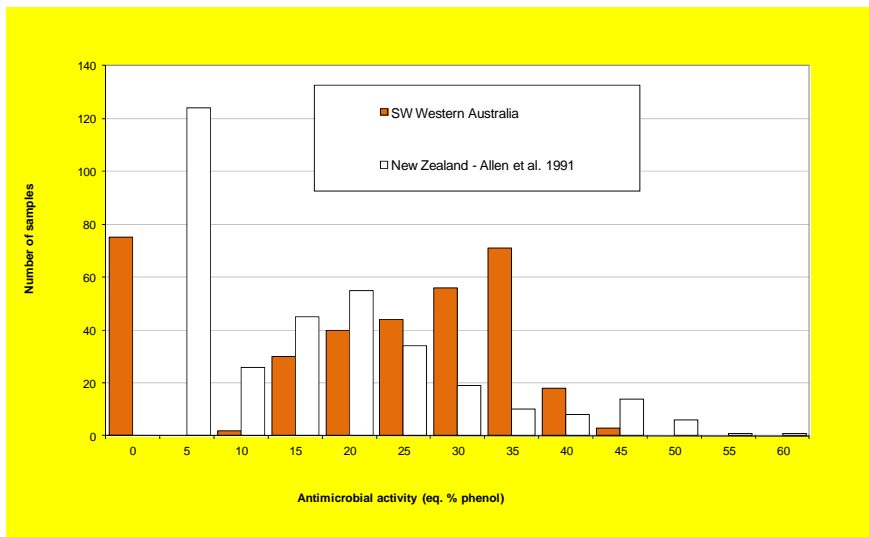


Fig. 2. The range of honey activity from WA and New Zealand honeys.

All Western Australian honeys surveyed were 'peroxide' honeys except two samples which were non-peroxide honeys: *Leptospermum subtenue* and a dark honey from the East Kimberley which subsequently tested extremely high for Zinc (570 mg/kg) and perhaps was responsible for its 'non-peroxide' reading.

The highest mean values of antimicrobial activity were for other eucalyptus species, in particular *Eucalyptus erythrocorys* Red-capped gum (36.7% w/v phenol), *E. diversicolor* Karri (32.2% w/v phenol), *E. patens* Forest blackbutt (31.1% w/v phenol), *E. occidentalis* Yate (30.5% w/v phenol) and *E. gomphocephala* Tuart (28.5% w/v phenol), *Eucalyptus wandoo* Wandoo (27% w/v phenol), *E. rudis* Flooded gum (27.9% w/v phenol), *E. platypus* Moort (0% w/v phenol), *E. flocktoniae* Merrit (0% w/v phenol), *E. loxophleba* York gum (0% w/v phenol) and *E. campaspe* Silver gimlet (16.7% w/v phenol). Other species results were: *Agonis flexuosa* Peppermint (0% w/v phenol), *Arctotheca calendula* Capeweed (0% w/v phenol), *Asphodelus fistulosus* Onion weed (27.7% w/v phenol), *Banksia grandis*

Bull Banksia (28.9% w/v phenol), *Brassica napus* Canola (14.7% w/v phenol), *Dryandra sessilis* Parrotbush (17.1% w/v phenol), *Echium plantagineum* Paterson's curse (15.3% w/v phenol), *Hakea trifurcata* Trifurcata (25.8% w/v phenol), *Helianthus annuus* Sunflower (0% w/v phenol), *Malus* sp Apple (0% w/v phenol), *Melaleuca* spp Paperbark (0% w/v phenol), *Nuytsia floribunda* Xmas tree (25.8% w/v phenol), *Prunus* sp Plum (0% w/v phenol), *Psoralea pinnata* Taylorina (12.6% w/v phenol).

There were 90 samples described as a mixture of species (24.4% w/v phenol) and 47 samples from species 'unknown' (19.8% w/v phenol). A commercial Medihoney™ sample measured 17.3% w/v phenol.

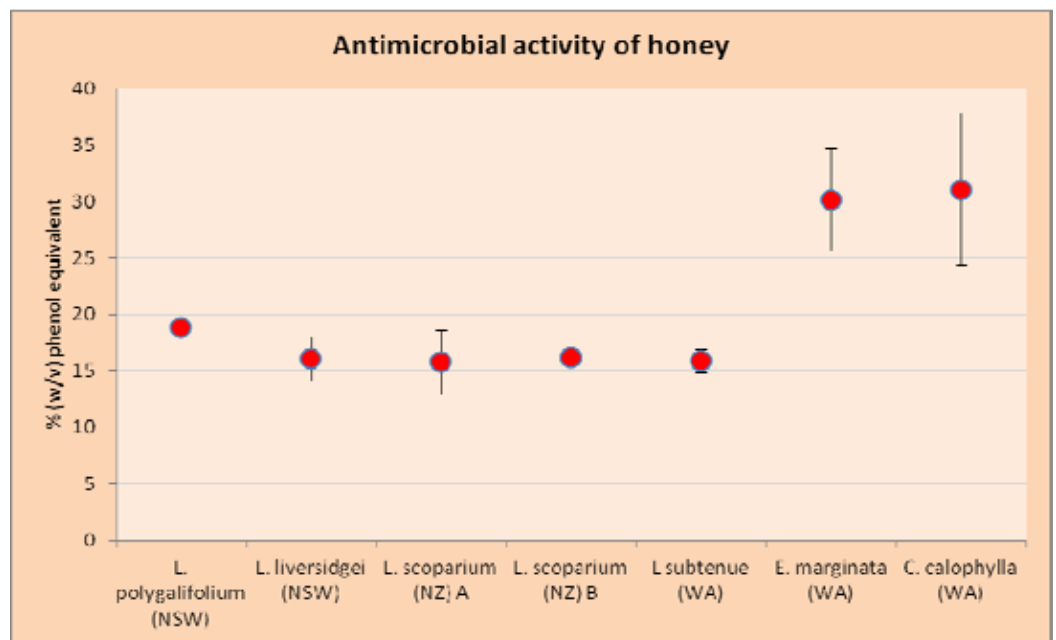


Fig 3. Comparison of antimicrobial activity of New Zealand's manuka honey and Australian 'manuka' species with Western Australian Jarrah (*Eucalyptus*) and Marri (*Corymbia*). Data (average, SE) from Irish *et al* (2011), Manning & Mercer (unpublished) and Allen *et al.* (1991).

Honeys gathered within the urban fringe of Perth where there is a huge diversity of plants grown in gardens, had a higher antimicrobial activity (19.2% w/v phenol) than Manuka (15.8% w/v phenol).

Antimicrobial activity in honey by year

Of all the honeys, Jarrah had the largest number of samples collected (N=61), which were used to determine whether significant year to year variations occurred in honey activity. The mean antimicrobial activity of Jarrah was compared over the years from 1999 to 2004, respectively (Fig 4). The results of a statistical ANOVA confirm that there was a significant difference ($P<0.0001$) between all the years.

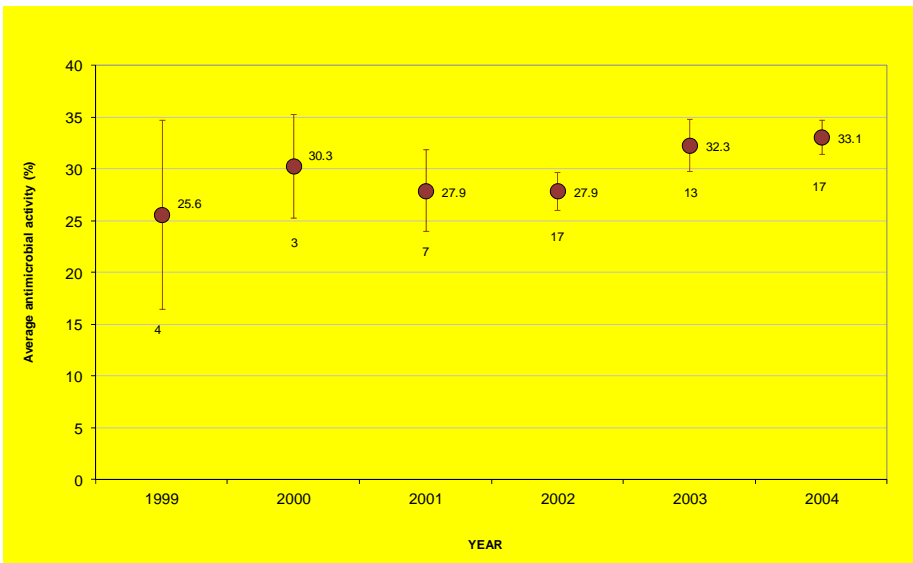


Fig 4. Yearly variation in Jarrah honey activity.

Exotic versus native plant-honey activity

There were 264 honey samples that could be separated into those honeys derived from exotic plants, those from native sources or that could be grouped as being a mixture of both.

An ANOVA of the mean antimicrobial values from the different sources showed significant differences between honey sources ($P<0.0001$). The main difference was the honey from native plants. An ANOVA test of the non-native honey sources showed no significant difference ($P=0.7923$) in the antimicrobial activity between exotic and mixed (native-exotic) honey sources.

Honeys from native plants had a mean antimicrobial activity significantly higher (27.6% w/v phenol) than that of all other sources. Native and exotic plant mixes were 18.3% w/v phenol. The foreign honeys (which were collected after being seized

following quarantine regulation breaches at the Perth airport) and honeys from exotic plants (mainly from farmland) had the lowest activity of 17.5% w/v phenol and 17.4% w/v phenol respectively.

Effect of minerals in honey on antimicrobial activity

Glucose oxidase is an enzyme added to the nectar by honey bees where it oxidises small amounts of glucose to gluconolactone which equilibrates with gluconic acid and helps stabilise the nectar processes against fermentation. For every molecule of glucose oxidized in the reaction, one molecule of hydrogen peroxide is produced which helps against microbial spoilage especially if the honey is diluted (White 1992, White *et al.* 1963 cited in Weston 2000). Research by Carter and Thornburg (2004) has shown plants such as tobacco, secrete five proteins into its nectar, one of which is glucose oxidase. As a result, high levels of hydrogen peroxide in the nectar could be generated to levels up to 4 mM.

The high level of hydrogen peroxide in some honeys could also be due to the lack of plant-derived catalase in the honey, which originates from pollen (Dustman 1971 cited by Weston 2000).

The presence of metal ions in the nectar can cause chemical reactions when hydrogen peroxide levels are high (Carter and Thornburg 2004). Therefore the mineral content of honey could influence the resultant presence of hydrogen peroxide.

Results from WA research show high levels of potassium, manganese, magnesium, copper and zinc are more likely to be associated with honeys antimicrobial activity whilst high levels of boron, sulphur, iron and calcium are more likely to be associated with a lack of antimicrobial activity.

Potassium and iron are the most important minerals for discriminating between honey samples with and without antimicrobial activity. Calcium and magnesium are the most important minerals in

separating honey derived from exotic or native plant species.

Of the macro-nutrients, potassium was the most dominant mineral in all honeys, whilst of the micro-nutrients, boron was highest in concentration in honeys from exotic plants (8.8 mg/kg) and manganese was highest in honey from native plants (5.4 mg/kg) - see Table 1.

Mineral	Exotic plant	Exotic-native mix	Native plant
% K	0.04	0.15	0.17
% Na	0.007	0.021	0.031
% Ca	0.005	0.010	0.017
% Mg	0.0024	0.0032	0.0090
% P	0.0046	0.0036	0.0039
% S	0.0039	0.0036	0.0043
B mg/kg	8.8	5.7	4.0
Mn mg/kg	0.7	0.6	5.4
Fe mg/kg	2.4	2.1	2.5
Zn mg/kg	1.0	1.2	2.0
Cu mg/kg	0.16	0.24	0.23

Correlation of antimicrobial activity with minerals

Correlation statistics which measure the strength of the linear relationship between two variables showed a high positive correlation with antimicrobial activity and potassium and manganese followed by magnesium, calcium and sodium. The only element to have a strong negative correlation with antimicrobial activity was boron (Table 2).

Activity	1.00											
P	-0.02	1.00										
K	0.59	0.09	1.00									
Na	0.20	-0.01	0.38	1.00								
Ca	0.34	-0.07	0.41	0.25	1.00							
Mg	0.40	0.02	0.50	0.27	0.85	1.00						
S	-0.03	0.18	0.12	0.44	-0.12	-0.07	1.00					
B	-0.51	0.07	-0.53	-0.38	-0.44	-0.50	-0.04	1.00				
Cu	0.20	0.20	0.46	0.36	-0.05	0.06	0.25	-0.18	1.00			
Fe	-0.01	0.01	0.23	0.10	0.04	0.05	0.05	0.10	0.27	1.00		
Mn	0.53	-0.15	0.42	0.06	0.74	0.71	-0.24	-0.52	-0.08	-0.05	1.00	
Zn	-0.12	0.18	0.02	0.44	0.19	0.21	0.24	-0.01	0.23	0.16	-0.01	1.00
	Activity	P	K	Na	Ca	Mg	S	B	Cu	Fe	Mn	Zn

In honey, calcium strongly correlated positively to magnesium and manganese. Boron had a weak negative relationship with antimicrobial activity,

potassium, magnesium and manganese. Magnesium had a weak positive relationship with potassium, manganese had a weak positive relationship with antimicrobial activity. Antimicrobial activity had a weak positive relationship with potassium.

Phosphorus, sodium, sulphur, copper, iron and zinc did not have a relationship with any other mineral tested.

Discussion

Of the species where five or more samples were collected, two species, Marri and Jarrah, had high average antimicrobial activity of 31.1% and 30.2% (w/v) phenol equivalent, respectively against *S. aureus*. The activity was significantly higher than New Zealand's manuka honey, which averaged 15.8% phenol which was similar to the average 16.2% activity measured by Allen *et al.* (1991). See Figure 2.

Jarrah has been tested against other microbes. Irish *et al.* (2006a) found unprocessed Jarrah honey was significantly more active against three *Candida* species than two medi-styled proprietary honey brands tested (one of which was Medihoney™, a blend of non-peroxide (manuka) honey) and a high-activity peroxide honey. Further work using Jarrah honey has also shown it to be an effective agent for preventing biofilm formation in

S. aureus in vitro and could be useful on particularly important medical devices such as catheters (Irish *et al.* 2006b).

More recently, Irish *et al.* (2011) published findings that showed both Marri and Jarrah's honeys total antimicrobial activity are the highest in Australia from a survey of 477 honeys. Their values given as a 'median' and not an average for Marri was 25.7% w/v phenol (8), Jarrah 25.1% w/v phenol (19) and Tasmanian Manuka (same species in NZ) 13.1% w/v phenol (11). These values **strongly** validate the earlier Western Australian survey results of Jarrah and Marri (Manning and Mercer unpublished).

For many of the other plant species in the original Manning & Mercer WA survey, Irish *et al.* (2011) also found low activity (median values) of Wandoo (<5% w/v phenol), *Eucalyptus accedens* Powderbark (<5% w/v phenol), Parrotbush (<5% w/v phenol), Paperbark (7.4% w/v phenol) and Paterson's curse (6.3% w/v phenol). Again a majority of these honeys had samples which had no activity (the exception was Powderbark). The <5 % value was categorized as 'undetectable' activity (Irish *et al.* 2011).

Of the two WA species, Marri and Jarrah, pure Jarrah characteristically remains in a liquid state for a protracted period of time which markedly differs to Marri in this respect. Jarrah honey's lack of crystallization (candying) was reported by Chandler *et al.* (1974). Honeys that solidify have to be reheated to return them to a liquid state. The heating process can destroy the enzyme, glucose oxidase that is linked to honeys antimicrobial activity. Other enzymes in honey such as diastase and invertase have been shown to remain active providing the honey is not heated above 45°C for 16 hours. Temperatures above 50°C generally cause rapid loss of activity (Langridge 1977). However, the activity does decrease over time. From Irish *et al.* (2011) research, peroxide honeys stored for 8 to 22 months lost an average 9.5% activity and the loss was significantly less if the product is stored at 4°C than 25°C.

Consumers benefit from honey being a liquid and the fact that Jarrah often grows in almost pure-species stands on laterite soils of the Darling Plateau (Wheeler and Byrne 2006) make Jarrah the preferred honey source as a functional food which could be exploited in a pharmaceutical market. Jarrah honey is harvested from September to

January and Marri is harvested from February to March (Smith 1969). Both species generally have a biennial flowering where a honey harvest can be equivalent to several hundred tones but the yield is significantly impacted by the environment (Fire, prescribed burns, drought (soil moisture) and high temperatures)

In the years since the discovery of the antimicrobial properties of WA honeys, the promotional push has been like that of New Zealand where a single species has been identified (e.g. Jarrah) and given a status amongst consumers. However, in WA a series of poor years of production from the Jarrah forest has meant there has been a shortage of this honey and like Manuka with its limited production volumes, mixes may have to become more common where another highly active honey could be blended into the dominant market species to resolve some of the problems of supply and demand (beyond the normal price increases). In the case of Jarrah, a Jarrah blend with Marri with its equivalent high activity seems an appropriate way to increase supply if candying problems are not created. The process of blending should be reflected in product labeling such as "Jarrah 80" keeping the dominant brand name in the label.

Due to poorer average antimicrobial activity from exotic honeys (usually harvested from agricultural plants) it is extremely important to maintain beekeepers access to native plant resources where the highest quality antimicrobial honeys exist. The government land agency is continually developing prime (and traditional) beekeeping land into conservation reserves and National Parks. Current policies tend to restrict beekeeping and should be carefully assessed because of the potential health benefits to the community of beekeepers having access to the resource. Mixtures of honey from both exotic and native sources gave activity readings which were not significantly different to the activity of exotic honeys which also supports the argument for apiary sites to be retained within a forest and not relocated to the boundaries of forest reserves which are adjacent to farmland.

Twenty percent of honey samples showed no antimicrobial activity and this was similar to that found from other surveys of honey e.g. Allen *et al.*

1991, Brady *et al.* 2004, Davis 2005 and Irish *et al.* 2011). Just why some honeys showed no activity is unknown. Dustman 1971 (cited in Weston 2000) suggested that the high level of hydrogen peroxide in some honeys could also be due to the lack of plant-derived catalase in the honey, which originates from pollen.



Fig 5. Post operative treatment of a skin graft with Jarrah honey. Using an application of honey + non woven Multigate sterile pad twice a day, the infected wound healed in 26 days.

From this, one could theoretically surmise that frames of honey from the brood chamber of bee hives, which can contain substantial amounts of pollen, might not be active whereas, honey from a queen-excluded honey super (a box above a brood chamber which sits above a metal or plastic device that prevents queen bee access i.e. no eggs and little requirement for pollen to be stored) would tend to be more active. Beekeeping businesses can operate apiaries with or without queen excluders and it is possible that samples of honey came from each hive

type. The theory could be tested in further experiments.

Western Australian antimicrobial activity results are far higher than the average 7.6% w/v phenol measured for Portuguese honey (Henriques *et al.* 2005). Three confirmed eucalypt honeys collected in Portugal showed surprisingly low activity at 6.8% w/v phenol when compared with our samples from the same genus. In a survey by Brady *et al.* (2004), a single *Eucalyptus* sample showed a higher value of 16.8% phenol. The differences may have been due to different *S. aureus* isolates used in the experiments, (though the isolate used by Brady *et al.* (2004) was identical), the way the honey was collected and stored or differences in the pH of the 10% phenol standard. For instance, a high pH value of 7.18 of a phenol batch (which should be pH 6) does cause a reduced zone diameter of the phenol standard which influences the final calculation for % (w/v) phenol. However, most of WA data has been confirmed by Irish *et al.* (2011) where Jarrah and Marri are two of the highest activity peroxide honeys in Australia and indeed perhaps the world.

The average antimicrobial activity can change on a year to year basis. Jarrah showed significant differences in antimicrobial activity between some years in inhibiting the growth of *S. aureus*. The honeys were collected from different sites in the forest because of Jarrah's biennial flowering, which can be a general flowering over the whole forest or one where beekeepers find nectar flows in discrete areas. DeMera and Angert (2004) noted that phylogeographically, botanical origin is influential on honeys antimicrobial activity.

Use of Jarrah honey

“There is a large body of evidence to support the use of honey as a wound dressing for a wide range of types of wounds. Its antibacterial activity rapidly clears infection and protects wounds from becoming infected, and thus it provides a moist healing environment without the risk of bacterial growth occurring. It also rapidly debrides wounds and removes malodor. Its anti-inflammatory activity reduces edema and exudate and prevents or minimizes hypertrophic scarring. It also stimulates the growth of granulation tissue and epithelial tissue so that healing is hastened. Furthermore, it creates a

nonadherent interface between the wound and the dressing so that dressings may be easily removed without pain or damage to newly regrown tissue.” says Dr Peter Molan (2006).



Fig 6. Treatment of an infected wound in a dog with Jarrah honey. Treated twice a day with Jarrah honey on a piece of dry paper towel over the wound, it healed in 15 days.

Jarrah (and Marri) honey is still considered to be a quality table honey. The medical aspects of Jarrah (and Marri) honey lie in treating surface wounds such as ulcers, skin grafts and cuts or burns. Anecdotal evidence of Jarrah use in these aspects can be seen in Figs 5 & 6. A benefit of using Jarrah honey in horse leg wounds (besides its healing capacity) was reported to being able to stop the use of expensive Solosite gel and Melolin pads on a wound two weeks post veterinary treatment

(Camarda 2007). A full review of honey and its effect on wounds by Dr Molan can be found on the Web by typing in the details of his 2006 paper (see references).

Antioxidants in Jarrah honey

Honeys are a valuable source of antioxidants. These chemicals like phenolic acids, flavonoids, carotenoids, ascorbic acid are a dominant pool of antioxidants that act synergistically and can explain many of the biological and therapeutic properties of honey.

Testing in Tasmania has shown Jarrah honey to have high antioxidant activity. The importance of antioxidants is that it may help protect against cellular damage and the development of chronic diseases. Jarrah honey is a dark amber honey with a high mineral content than most other honeys. Its dark colour reflects the content of pigments with antioxidant properties. Close correlations of honey colour and phenol content, colour and FRAP and ORAC values of 0.93, 0.92 and 0.73 respectively, can reflect honeys' antioxidant capacity (Beretta *et al.* 2005). Antioxidants are measured in three types of assays:

1. Oxygen radical absorbance capacity (ORAC) also known as antilipoperoxidant activity³,
2. Ferric reducing ability of plasma (FRAP),
3. 2,2-diphenyl-1-picrylhydrazyl (DPPH), also known as antiradical activity,

All the values of the assays give total activity⁴.

Buckwheat honey has one highest antioxidant levels in the world⁵, where it can increase blood serum antioxidant capacity. For Tasmanian Buckwheat samples, total antioxidant activity is 16.93 TE $\mu\text{mol/g}$. This is compared to Manuka (NZ) at 17.6 TE $\mu\text{mol/g}$ and Jarrah 12.81 TE $\mu\text{mol/g}$.

³ TE as the final value is expressed as μmol Trolox equivalent (TE)/g.

⁴ There is a linear correlation (0.87, 0.96) with phenolic content & ORAC; between DPPH & FRAP values (0.85), phenolic content and DPPH (0.92) and phenolic content and FRAP (0.89).

⁵ Honey from *Arbutus unedo* (Strawberry tree) is higher (Beretta *et al.* (2005) at 21.07 TE $\mu\text{mol/g}$

Lighter honeys (400-500 mAu/cm) have activity levels of 6.1 to 9.61 TE $\mu\text{mol/g}$. Jarrah honey measures⁶ 946 mAu/cm in colour and is darker than Manuka (932 mAu/cm) and Buckwheat (900 mAu/cm).⁷

The phenolic content, which is a laboratory index of antioxidant strength, shows Jarrah honey is higher (207.4 mg gallic acid/kg) than Tasmanian grown Buckwheat⁸ (*Fagopyrum esculentum*) (200.3 mg gallic acid/kg) or Manuka (197.4 mg gallic acid/kg). There results of phenolic content with assay values from FRAP, DPPH and ORAC are highly correlated and this indicates that the antioxidant capacity of honey is due to their phenolic constituents (Beretta *et al.* 2005).

Honey sugars

Forest Jarrah honey is a high fructose honey with fructose, glucose and sucrose levels of 51.7%, 22.4% and 0.3% respectively and is lower for sandplain Jarrah 44.3%, 26.6% and 3.8%. Other WA species sugar compositions are:

Marri-Forest blackbutt is 40.5% 30.9% and 4.6%,
Marri-Karri average is 44.3%, 29.5% and 1.9%

Parrotbush 43.2%, 30% and 0%.

Calothamnus sanguineus (Red bell) 37.2%, 26% and 10.1%.

Banksia grandis 36.9%, 23.1% and 15.3%

Banksia menziesii 34.9%, 28.4% and 13.8%

Xanthorrhoea preissii (Grasstree) 32%, 22.2% and 19.3% (Chandler *et al.* 1974). The latter four species have naturally high levels of sucrose and this was discovered after Japan rejected WA honey shipments in 1964 for breaching their 5% sucrose specification. Thinking honey had been adulterated with sugar, research by Dr Francis Smith (Smith 1965) confirmed the high levels of sucrose were naturally occurring.

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⁶ Colour is expressed as mAu/cm for 50% (w/w) honey solutions.

⁷ 2009 data from Novost Pty Ltd (7 Wendover Place, Newtown, Tasmania 7008).

⁸ Compares with Californian buckwheat honey (482 mg gallic acid/kg); Mexican buckwheat (456 mg gallic acid/kg) (Beretta *et al.* 2005).

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A Jarrah tree in full blossom.