Chapter Three: Results

3.1 Antioxidant properties of bee products.

3.1.1 Ten fold dilutions.

Table 1. Mean FRAP values (µmol/l) of products and pure compounds.

	100 mg/ml	10 mg/ml	1.0 mg/ml	0.1 mg/ml
Propolis Resin	*N/A*	*N/A*	746 ± 20*	95 ± 5
Propolis Tablet	*N/A*	*N/A*	95 ± 0*	26 ± 3
Bee Pollen	706 ± 15	140 ± 0	15 ± 8	5 ± 0
Royal Jelly	773 ± 30	100 ± 0	8 ± 2	5 ± 0
Manuka 30+	306 ± 23	100 ± 0	9.0 ± 0	0 ± 0
Tea tree 18+	210 ± 0	27 ± 18	0 ± 0	0 ± 0
Tea tree 16+	206 ± 66	0 ± 0	0 ± 0	0 ± 0
Tea tree 12+	298 ± 88	0 ± 0	0 ± 0	0 ± 0
Manuka 5+	346 ± 3	33 ± 3	5 ± 0	3 ± 2
Standard Honey	165 ± 5	25 ± 0	5 ± 0	0 ± 0
Quercetin	*N/A*	*N/A*	*N/A*	793 ± 25
Cinnamic Acid	*N/A*	616 ± 70 *	41 ± 38	0 ± 0
Naringenin	*N/A*	280 ± 45 *	0 ± 0	0 ± 0

Artificial honey did not produce a FRAP reading.
N/A indicates the solution was too dark to obtain a FRAP reading.
* = significant results ($p<0.05$)
Dilutions 0.1mg/ml, 0.01mg/ml, 0.001mg/ml: post hoc tests could not be performed as too many samples had a FRAP reading of zero. Therefore, significance of results at these dilutions could not be determined.

Table 2. Comparison of mean FRAP values of bee products and pure compounds at 1.0 mg/ml.

Product	Mean FRAP value (µmol/l)
Quercetin	8000
Propolis Resin	746
Propolis Tablet	95
Cinnamic Acid	41
Naringenin	31
Bee Pollen	15
Manuka 30+	9.0
Royal Jelly	8.0
Manuka 5+	5.0
Standard Honey	5.0
Tea tree 12+	2.4
Tea tree 18	2.4
Tea tree 16+	1.8

The figures highlighted in red in Table 2 represent extrapolated data calculated using values obtained at alternate dilutions to allow comparison of the test compounds/bee products (assumes correlation between dilutions and FRAP values). The table is to illustrate which compounds have the highest and lowest antioxidant activity overall.

3.1.2 Percentage Dilutions

Table 3. Mean FRAP values (µmol/l) of honeys at different dilutions.

	50%	**25%**	**12.5%**	**6.25%**	**3.125%**
Standard Honey	808 ± 83	333 ± 32	0 ± 0	0 ± 0	0 ± 0
Manuka 5+	*N/A*	640 ± 10*	353 ± 5	165 ± 5	90 ± 8
Tea tree 12+	*N/A*	606 ± 11	343 ± 25	188 ± 36	75 ± 8
Tea tree 16+	816 ± 15	440 ± 10	203 ± 11	113 ± 5	83 ± 12
Tea tree 18+	*N/A*	608 ± 10	346 ± 5	186 ± 11	70 ± 15
Manuka 30+	*N/A*	776 ± 15*	556 ± 15*	195 ± 40*	158 ± 7*

Artificial honey did not produce a FRAP reading.
N/A indicates the solution was too dark to obtain a FRAP reading.
* = significant results ($p<0.05$)

Figure 3. Mean FRAP values of honeys at 25% dilution.

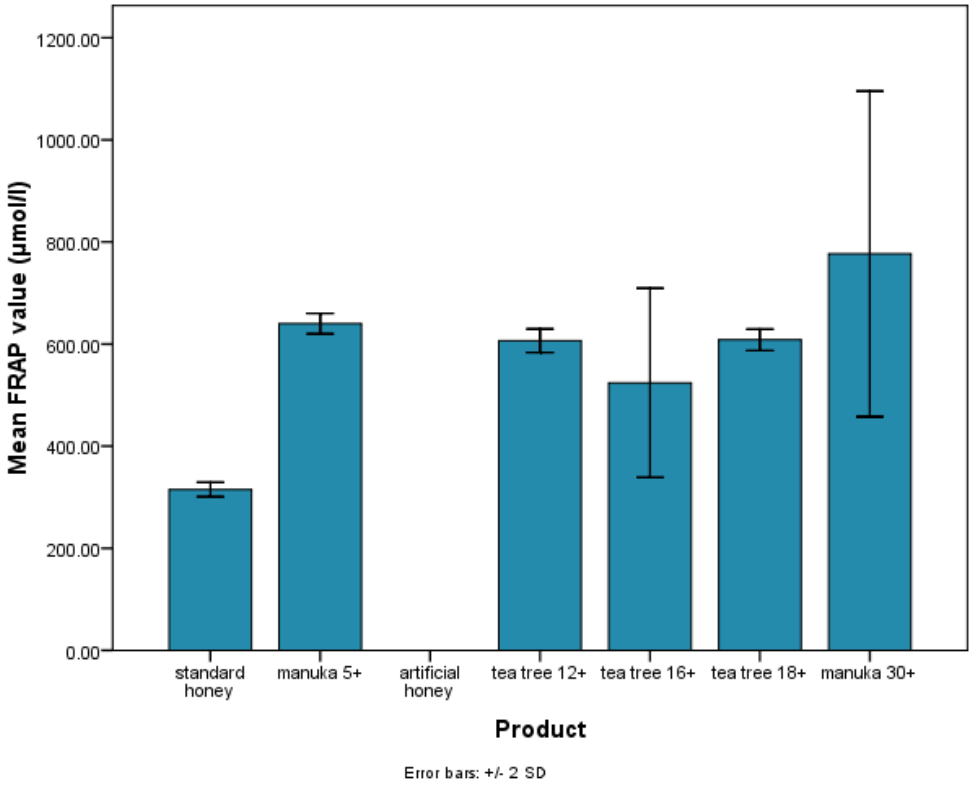


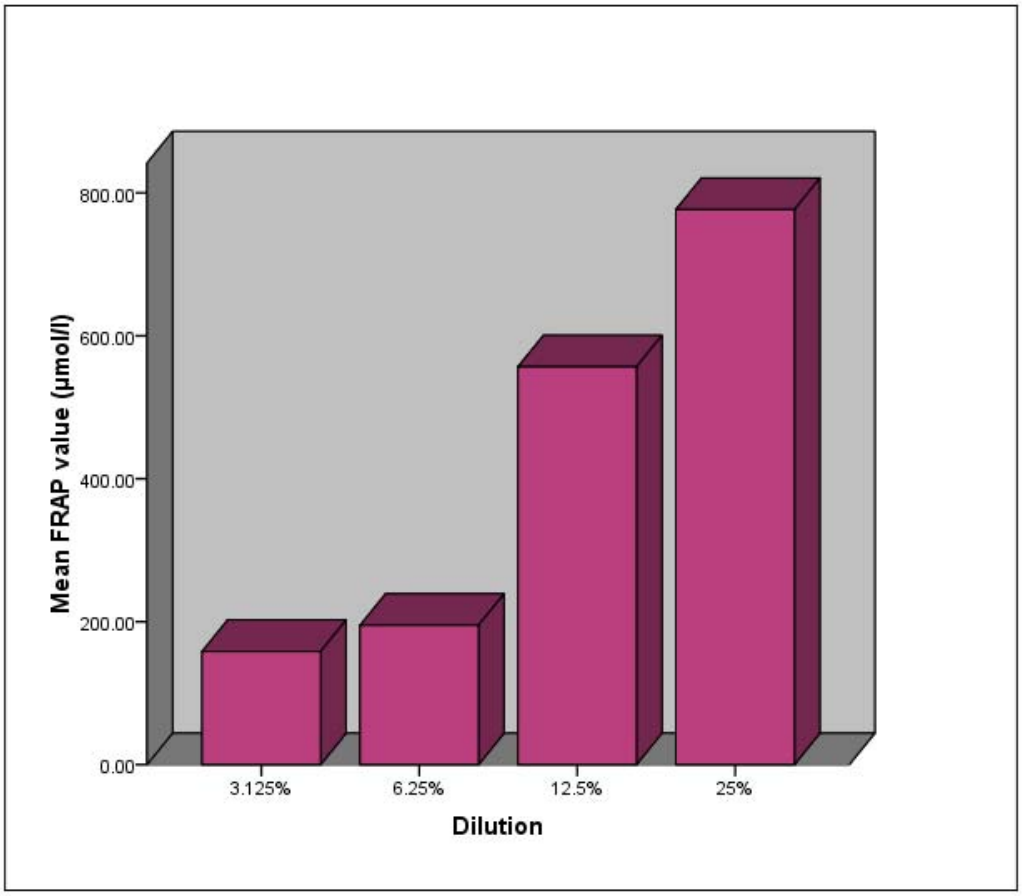
Table 4. Antioxidant activity (FRAP values) of honeys at different dilutions.

Order of antioxidant potency (FRAP value)						
Dilutions	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**
25%	**M30+**	**M5+**	**TT16+**	**TT18+**	**TT12+**	**Standard Honey**
12.5%	**M30+**	**TT16+**	**M5+**	**TT18+**	**TT12+**	**Standard Honey**
6.25%	**M30+**	**TT12+**	**TT18+**	**M5+**	**TT16+**	**Standard Honey**
3.125%	**M30+**	**M5+**	**TT16+**	**TT12+**	**TT18+**	**Standard Honey**

KEY: M30+ = manuka 30+, M5+ = manuka 5+, TT12+ = tea tree 12+, TT16+ = tea tree 16+, TT18+ = tea tree 18+.

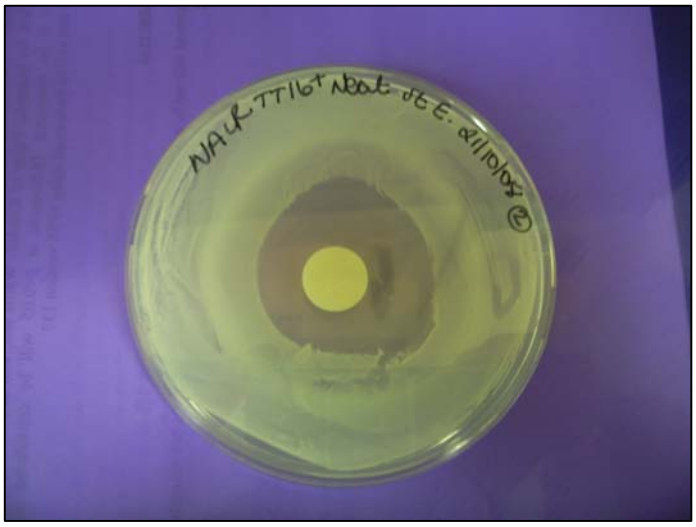
In Table 4 manuka 30+ had the highest mean FRAP value at every dilution and the standard honey had the lowest. The order of the other honeys varied with each dilution.

Figure 4. Dose response of mean FRAP values of manuka 30+.



3.2 Antibacterial properties of bee products using disc diffusion assay.

3.2.1 Photographs

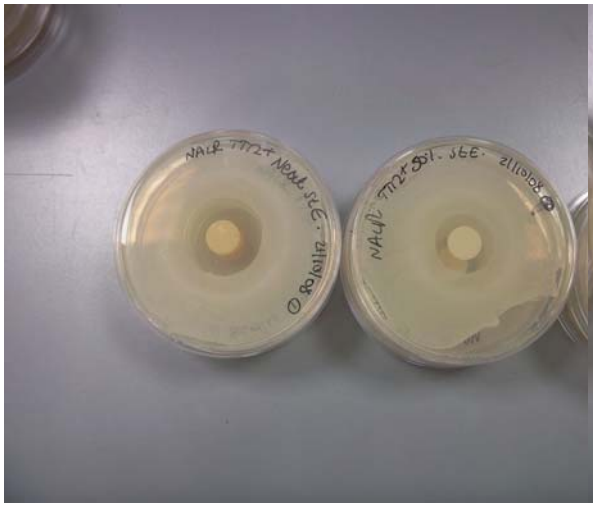


Photograph 1

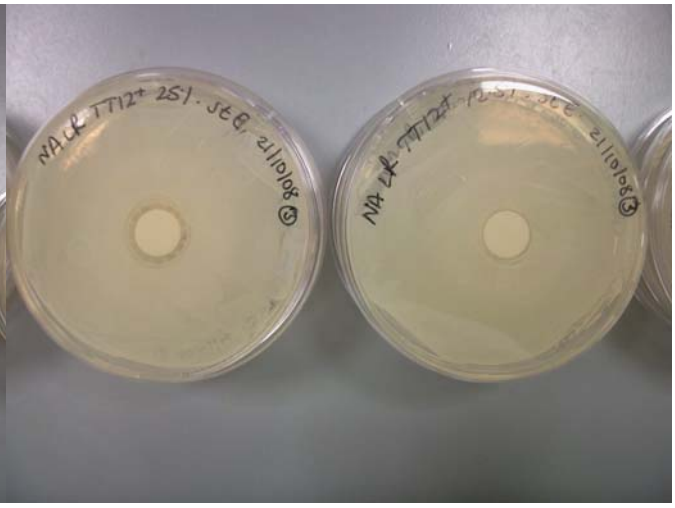
Photograph 2

Photograph 1 shows a large zone of inhibition around a disc dipped in neat 16+ tea tree honey added to a dish containing a *S.epidermidis* lawn.

Photograph 2 shows a plate with no zone of inhibition (manuka 5+ against *S. epidermidis*) next to a plate with a clear zone of inhibition (manuka 30+ against *S. epidermidis*).

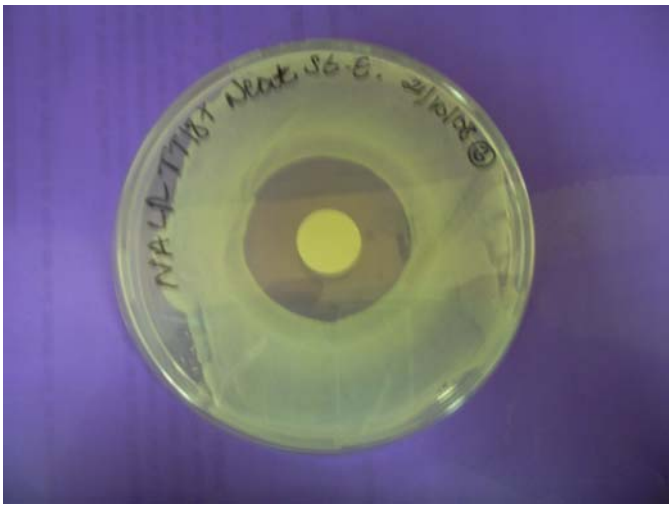


Photograph 3



Photograph 4

Photographs 3 and 4 show the decrease in diameter of zones of inhibition as the honey the discs are dipped in becomes more dilute.



Photograph 5

Photograph 5 shows a clear zone of inhibition. The plate had a *S. epidermidis* lawn and the disc was dipped in neat tea tree 18+ honey.

Table 5. Mean Zones of inhibition (mm) of bee products against *E.coli* using percentage dilutions.

	SH	M5+	TT12+	TT16+	TT18+	M30+	AH
Undiluted	0 ± 0	34 ± 4 *	10 ± 4	11 ± 2	12 ± 1	13 ± 6	34 ± 7*
50%	0 ± 0	19 ± 2*	4 ± 3	5 ± 1	9 ± 1*	0 ± 0	0 ± 0
25%	0 ± 0	7 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Key: M30+ = manuka 30+, M5+ = manuka 5+, TT12+ = tea tree 12+, TT16+ = tea tree 16+, TT18+ = tea tree 18+, SH= standard honey, AH= artificial honey. * = significant result ($p<0.05$).

When applied undiluted, the mean zones of inhibition of manuka 5+ and artificial honey were significantly higher than tea tree 12+, tea tree 16+, tea tree 18+ and manuka 30+ ($p<0.05$).

Table 6. Mean Zones of inhibition (mm) of bee products against *S.aureus* using percentage dilutions.

	SH	M5+	TT12+	TT16+	TT18+	M30+	AH
Undiluted	7 ± 6	4 ± 0	14 ± 1	17 ± 2*	18 ± 1*	24 ± 6*	3 ± 0
50%	21 ± 0*	0 ± 0	7 ± 1	9 ± 2	14 ± 1*	10 ± 2*	0 ± 0
25%	15 ± 1*	0 ± 0	0 ± 0	0 ± 0	8 ± 1	8 ± 3	0 ± 0
12.5%	8 ± 1*	0 ± 0	0 ± 0	0 ± 0	4 ± 1	0 ± 0	0 ± 0

Key: M30+ = manuka 30+, M5+ = manuka 5+, TT12+ = tea tree 12+, TT16+ = tea tree 16+, TT18+ = tea tree 18+, SH= standard honey, AH= artificial honey. * = significant result ($p<0.05$).

A decrease in the zones of inhibition was observed for all the honeys, except standard honey which had higher mean zones of inhibition (mm) at 50%, 25% and 12.5% compared with undiluted (Table 6). When applied undiluted, the mean zone of inhibition of manuka 30+ was significantly higher than that of tea tree 12+ ($p<0.05$). When applied

undiluted, the mean zone of inhibition of tea tree 16+ and tea tree 18+ was significantly higher than that of tea tree 12+ , manuka 5+ and standard honey ($p<0.05$).

Table 7. Mean Zones of inhibition (mm) of bee products against *S.epidermidis* using percentage dilutions.

	SH	M5+	TT12+	TT16+	TT18+	M30+	AH
Undiluted	11 ± 4*	6 ± 1	19 ± 2*	22 ± 1*	20 ± 2*	33 ± 5*	3 ± 0
50%	23 ± 1*	0 ± 0	11 ± 3	12 ± 2	15 ± 1*	8 ± 3	0 ± 0
25%	13 ± 2*	0 ± 0	4 ± 1	5 ± 1	8 ± 1*	0 ± 0	0 ± 0

Key: M30+ = manuka 30+, M5+ = manuka 5+, TT12+ = tea tree 12+, TT16+ = tea tree 16+, TT18+ = tea tree 18+, AH= artificial honey

When applied undiluted, the mean zone of inhibition of manuka 30+ was significantly higher than that of artificial honey, standard honey, manuka 5+, tea tree 12+, tea tree 16+, tea tree 18+ ($p<0.05$). When applied undiluted, the mean zone of inhibition of tea tree 16+ was significantly higher than that of artificial honey, standard honey, manuka 5+ ($p<0.05$).When applied undiluted, the mean zone of inhibition of tea tree 18+ was significantly higher than that of artificial honey, standard honey, manuka 5+ ($p<0.05$).

When applied undiluted, the mean zone of inhibition of tea tree 12+ was significantly higher than that of artificial honey, standard honey, manuka 5+ ($p<0.05$). When applied undiluted, the mean zone of inhibition of standard honey was significantly higher than that of artificial honey ($p<0.05$)

Table 8. Mean Zones of inhibition (mm) of bee product against *S.mutans* using percentage dilutions.

	SH	M5+	TT12+	TT16+	TT18+	M30+	AH
Undiluted	0 ± 0	0 ± 0	11 ± 2	21 ± 2*	31 ± 3*	19 ± 4*	0 ± 0
50%	0 ± 0	0 ± 0	0 ± 0	6 ± 1	15 ± 1*	5 ± 0	0 ± 0
25%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	7 ± 1	0 ± 0	0 ± 0

Key: M30+ = manuka 30+, M5+ = manuka 5+, TT12+ = tea tree 12+, TT16+ = tea tree 16+, TT18+ = tea tree 18+, AH= artificial honey

When applied undiluted, the mean zone of inhibition of tea tree 18+ was significantly higher than that of tea tree 12+, tea tree 16+ and manuka 30+ ($p<0.05$).When applied undiluted, the mean zone of inhibition of tea tree 16+ was significantly higher than that of tea tree 12+ ($p<0.05$).When applied undiluted, the mean zone of inhibition of manuka 30+ was significantly higher than that of tea tree 12+ ($p<0.05$).

For all the bacteria tested no measurable zones of inhibition were observed when using propolis tablet, cinnamic acid, naringenin or quercetin.