Chapter One: Introduction

1.1 General Introduction

There is considerable interest in the role free radical mediated damage may play in the development of degenerative diseases. Evidence has suggested reactive free radicals are involved in the development of heart disease and cancer (Garcia, de Pascual, Santos & Rivas, 2004).

Free radicals are highly reactive molecular species due to one or more unpaired electrons. Oxygen is a major source of reactive oxygen species (ROS) (Stephens, Khanolkar & Bain, 2009). ROS and reactive nitrogen species (RNS) are normally formed accidentally in metabolism or deliberately by cells as a defence against invading microorganisms (Cornelli, 2009).

Oxidative stress (OS) results from an imbalance between oxidant production and antioxidant defences. OS may be temporary but can damage proteins, lipids and DNA. Illnesses such as cardiovascular disease (CVD), cancers, neurological and endocrinological disorders have been linked with OS as either a cause or consequence of the disease. Increased OS is associated with some of the risk factors implicated in the pathophysiology of atherosclerosis such as diabetes mellitus, hypercholesterolaemia, renal failure, ageing, hypertension and smoking (Stephens et al, 2009). Additional ROS formed in cells include the superoxide anion ($O^{2-\bullet}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^{-\bullet}$) and the peroxynitrite radical ($OONO^-$).

Many chemical entities may have direct or indirect antioxidant activity. A dietary antioxidant is a substance in food that significantly decreases the adverse effects of (ROS), (RNS) or both on normal physiological function in humans (Food and Nutrition

Board, 2002, as cited by Cornelli, 2009). According to Cornelli (2009), an appropriate equilibrium between oxidation and antioxidants is fundamental to life. Endogenous antioxidant systems exist to reduce oxidative stress. These include: vitamins (A, C & E); enzymes such as superoxide dismutase (SOD); and non nutrient components such as flavonoids and carotenoids (Stephens et al, 2009).

Flavonoids (Figures 1 & 2) are members of a class of natural compounds that has been the subject of considerable scientific and therapeutic interest. Recent evidence has indicated flavonoids have an important effect on the intricate regulatory processes disturbed by cardiovascular disease, the most frequent cause of death in the developed world (Havsteen, 2002). Flavonoid toxicity to animal cells is low, however they can inhibit or kill many bacterial strains, inhibit viral enzymes and destroy some pathogenic protazoans *in vitro*. There is an increase in the use of flavonoids in the treatment of diseases due to their ability to inhibit specific enzymes and scavenge free radicals (Havsteen, 2002).

Figure 1. The generic structure of flavonoids.

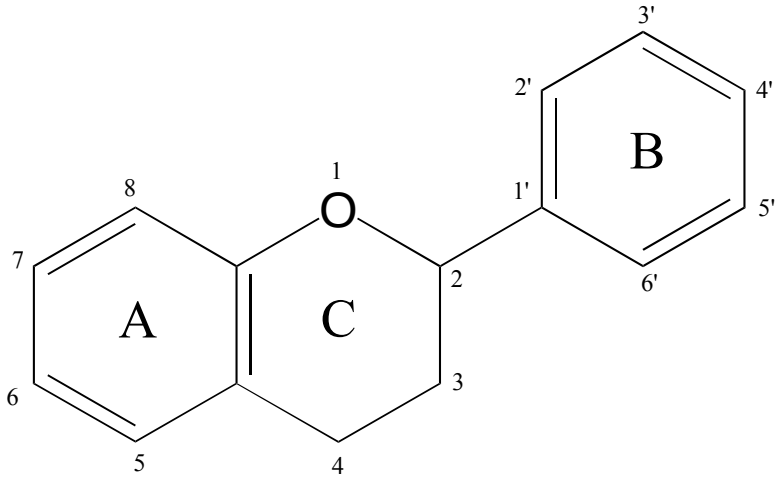
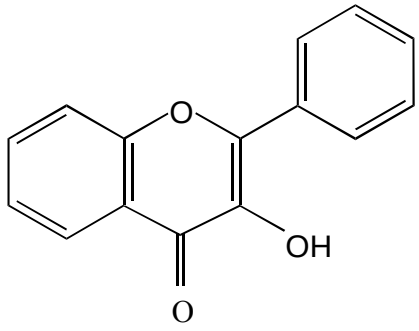
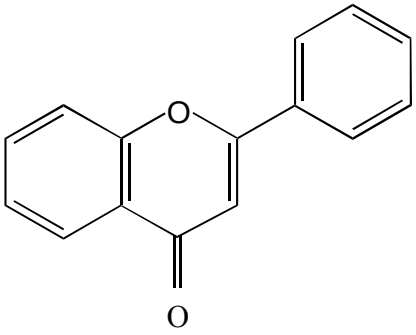


Figure 2. Structure of the main subclasses of flavonoids.

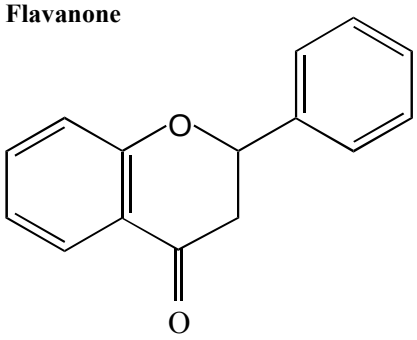
Flavonol



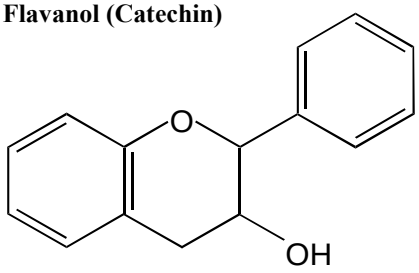
Flavone



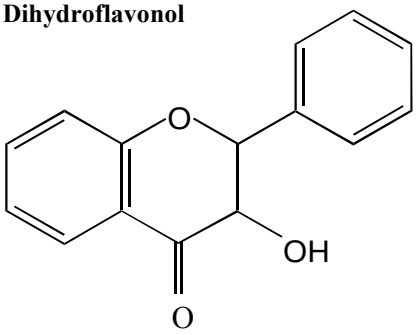
Flavanone



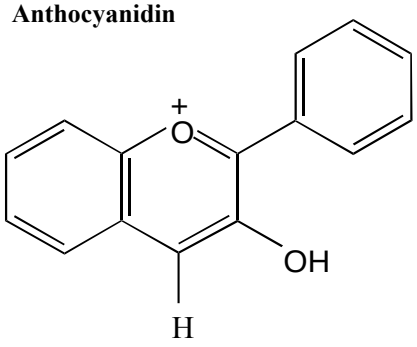
Flavanol (Catechin)



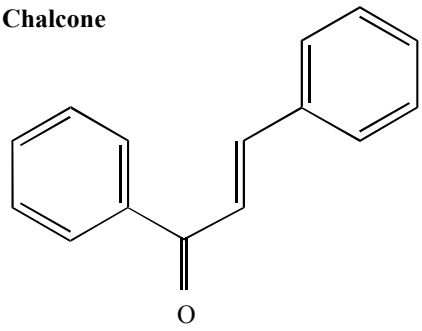
Dihydroflavonol



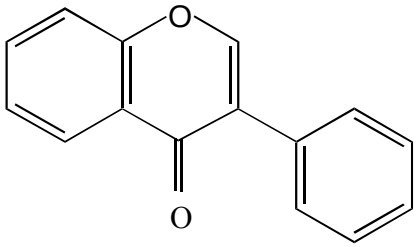
Anthocyanidin



Chalcone



Isoflavone



The role of antioxidants in protecting tissue from oxidative damage has had increased interest over recent years. A diet rich in fruits and vegetables containing various classes of polyphenols (phenolic acids, flavonols, catechin monomers, proanthocyanidins, flavones, flavanones, anthocyanins) decreases the risk of premature mortality from major clinical conditions, including cancer and heart disease (Duthie, Gardner, & Kyle, 2003).

Functional foods or nutraceuticals contain one or more components which positively and specifically promotes a physiological or psychological effect over and above the foods' traditional nutritive value (Viuda-Martos, Ruiz-Navajas, Fernández-López and Pérez-Álvarez, 2008). In recent years concerns regarding health has led to increased interest in functional foods by consumers, specifically antioxidant properties and potential contribution of functional foods in the prevention of diseases such as cancer and cardiovascular disease (Viuda-Martos et al., 2008).

1.2 Bee products

Bee products including honey and propolis are considered functional foods due to their naturally high antioxidant potential. Propolis is a resinous substance collected by honey bees from leaf buds and bark of plants. The bees mix the original propolis with beeswax and β- glucosidase secreted from their hypopharyngeal glands. The resulting material is used by bees to seal holes in the hive, exclude draughts, protect against external invaders and mummify their carcasses (Pietta, Gardana & Pietta, 2002). The word propolis is derived from the Greek pro, for or in defence, and polis, the city (defence of the hive) (Ghisalberti as cited by da Costa & Pereira, 2002). Propolis has been used as a medicine

as early as 300 BC and has been used extensively in folk medicine for many years. Current applications of propolis include over the counter preparations for upper respiratory infections, common cold and flu as well as dermatological preparations useful in wound healing, treatment of burns, acne, herpes simplex and genitalis and neurodermatitis (Burdock as cited by Pietta et al, 2002). As a result of this wide range of biological activities, propolis can now be found in a range of health food supplements and beverages. (Burdock as cited by Daugsch et al, 2007).

1.2.1 Propolis

Propolis has been shown to comprise of over 150 constituents including flavonoids such as naringenin and quercetin as well as other phenolic compounds such as cinnamic acid. The exact composition of propolis varies according to its variety and geographical source (Mishima, Yoshida, Akino & Sakamoto, 2005). Generally pharmacologically active molecules in propolis are flavonoids and phenolic acids and their esters (Castaldo & Capasso, 2002). Flavonoids are thought to be responsible for antibacterial, antifungal and antiviral actions of propolis (Ghisalberti as cited by Kujumgiev, Tsetkova, Serkedjieva, Bankova, Christov & Popov 1999). Esters of phenolic acids, caffeates and ferulates have been identified as antibacterial, antifungal and antiviral principles of propolis (Kujumgiev et al, 1999).

1.2.1.1 Antioxidant properties of propolis

Studies of propolis from Eastern Europe and South America have indicated flavonoids concentrated in propolis are powerful antioxidants (Banskota, Tezuka, Adnyana, Midorikawa, Matsushige, Message et al, 2000). Mohammadzadeh, Sharriatpanahi, Hamedi, Amanzadeh, Ebrahimi and Ostad (2007) investigated the antioxidant activity of Iranian propolis using the FRAP assay and found Tehran propolis ethanolic extract exhibited the highest FRAP value (1650μmol/l at concentration of 2mg/ml), however, no statistical significance was found between FRAP values of Tehran propolis ethanol extract and Trolox at concentration of 100μg/ml ($p>0.05$). The study also did not state if any statistically significant difference was found between the FRAP values of the three different propolis ethanolic extracts. The study proposed that strong antioxidative activity occurs in propolis with high amounts of phenolic compounds and weak activity with low amounts.

Kumazawa, Hamasaka and Nakayama (2004) investigated the antioxidant activity of propolis from various geographic origins. The study focused on the *in vitro* antioxidant activity of the ethanol extracts of propolis from various countries and analysed the individual constituents in the extracts. Several compounds in each extract were identified by HPLC analysis and their free radical scavenging activity measured. These researchers found a correlation between total flavonoid contents of the extracts and antioxidant activity. These propolis extract samples were found to have high total polyphenol and flavonoid contents. The study also found Chinese propolis had strong DPPH free radical scavenging activity whereas the activity of extracts from Brazil was weak. However, Alencar and Moura (2000) as cited by Kumazawa et al. (2004) reported the antioxidant

activity of propolis can not only differ between countries but also between different regions within the same country.

Kumazawa et al. (2004) proposed that although flavonoids are reported to be the most abundant and most effective antioxidants in propolis, other factors also contribute to its antioxidant properties. Kumazawa et al. (2004) did not state if any of the results obtained were statistically significant, additionally, the results would only have related to propolis from specific regions of the different countries; for example Brazilian propolis was found to have week DPPH free radical scavenging activity but several antioxidant compounds have been previously isolated from Brazilian propolis.

A study by Ahn, Kumazawa, Usui, Nakamura, Matsuka, Zhu et al. (2007) evaluated the antioxidant activity and constituents of ethanol extracts of propolis collected in various areas of China using three assay systems: the inhibition of linoleic acid oxidation by β-carotene bleaching, the free radical-scavenging activity on 1,1-diphenyl-2-picrylhydrazyl- (DPPH), and the scavenging activity on 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation. Ahn et al. (2007) found all, except one, propolis extract samples had high total poyphenol and flavonoid contents and the correlation between total polyphenol content and antioxidant activity was significant ($r^2 = 0.671$). Further positive correlations between extracts and total polyphenol contents were observed ($r^2 = 0.762$) and between ABTS radial cation scavenging activity of various extracts and total polyphenol contents ($r^2 = 0.459$).

1.2.1.2 Antimicrobial properties of propolis

Studies have demonstrated a synergistic effect of propolis on the anti-bacterial activity of antibiotics such as streptomycin and cloxacillin, and a moderate synergistic effect on the anti-bacterial activity of chloramphenicol, cefradine and polymyxis B in culture medium containing a fixed amount of a standard strain of *Staphylococcus aureus* (Krol, Scheller, Shani, Pietsz, Czuba & Arzneim-Forsch, 1993, as cited by Castaldo & Capasso, 2002).

The antimicrobial activity of ethanolic extracts of propolis against 140 Gram positive *Staphylococcus spp*. and 123 *Streptococcus* spp. strains was investigated by Scazzochio, D'Auria, Alessandrini and Pantanella (2006). The minimal inhibitory concentrations (MICs), defined as the lowest concentration to completely inhibit growth of bacteria, was determined for samples of propolis extracts and antibacterial drugs. The study found propolis was effective against many microorganisms, and sub-inhibitory concentrations of propolis extracts displayed a synergistic effect with many antibiotics. Although the study did not produce any statistically significant results, Scazzochio et al. (2006) suggested components in the propolis extracts such as flavonoids (quercetin, galangin, pinocembrin), caffeic acid, benzoic acid and cinnamic acid may damage the microbial membrane of cell wall site leading to function and structural damages. The observations made were *in vitro*; no *in vivo* synergy between propolis and antibiotics was reported. Scazzochio et al. (2006) proposed adding propolis to antibiotics may increase efficacy potentially reducing economical problems relating to antibiotic resistance.

Propolis has also shown an *in vitro* antibacterial activity against 15 bacterial strains clinically relevant to oral health (Koo, Gomes, Rosalen, Ambrosano, Park& Cury, 2000,

as cited by Castaldo & Capasso, 2002). Recent advances in understanding the aetiology and pathogenesis of periodontitis have led to improved pharmacological interventions. For example, medications can be placed directly into the periodontal pockets to suppress or eradicate the pathogenic microbionta or modulate the inflammatory response leading to a decrease in tissue destruction (Bollen & Quirynen, 1996; Greenstein & Polson, 1998 as cited by Sonmez, Kirilmaz, Yucesoy, Yücel & Yilmaz, 2005).

An investigation by Kujumgiev, Tsvetkova, Seredjeva, Bankova, Christov & Popov (1999) examined the antibacterial activity of propolis against *Stapylococcus aureus (S.aureus)* and *Escherichia coli (E.coli)*; antifungal activity on *Candida albicans* and antiviral activity on avian influenza. The study found all samples were active against the fungal and Gram positive bacterial test strains and most showed antiviral activity. No activity was found against the Gram negative *E.coli.* A study by Koo, Gomes, Rosalen, Ambrosano, Park and Cury (2000) investigated the antimicrobial actions of propolis against 15 oral microorganisms including *Staphylococcus aureus* and *Streptococcus mutans*. The researchers found the ethanolic extract of propolis produced inhibitory zones against all microorganisms, including periodontopathogenic anaerobes. Growth of the bacteria were also significantly inhibited by the propolis extracts ($p<0.05$).

1.2.2 Honey

Apitherapy, treatment with natural honey, has been used by many different cultures throughout history. The therapeutic potential of honey is gradually growing and honey has been reported to be effective in gastrointestinal disorders, wound healing and as an anti-microbial agent (Al-Mamary, Al-Meer & Al- Habori, 2002).

Honey is composed of at least 181 components and is a solution supersaturated with sugars: fructose (38%) and glucose (31%) are considered the most important (Gheldof, Wand & Engeseth, 2002 as cited by Viuda-Martos et al., 2008). The moisture content is approximately 17.7%, total acidity 0.08% and ashes 0.18% (Nagai, Inoue, Kanamori, Suzuki & Nagashima, 2006). Additional components include phenolic acids and flavonoids, enzymes glucose oxidase and catalase, ascorbic acid, carotenoids, organic acids, amino acids, proteins and α-tocopherol (Ferreres, Garciaviguera, Tomaslorente & Tomasbarberan, 1993, as cited by Viuda-Martos et al., 2008). The actual composition of honey varies depending on pollen source, climate, environmental conditions and processing(Gheldof et al., 2002; Azeredo, Azeredo, de Souza & Dutra, 2003 as cited by Viuda-Martos et al., 2008). According to Weston (2000); raw honey contains many compounds including flavonoids and other polyphenols, which may function as effective natural antioxidants.

1.2.2.1 Antioxidant properties of honey

Blasa, Candiralli, Accorsi, Pier-Piacentini, Albertini and Piatti (2006) used the Ferric-reducing antioxidant power (FRAP) assay to determine the antioxidant power of two common varieties of Italian honey, Millefiori and Acacia, compared with an artificial

honey (represents sugar content of honey). The study found the Millefiori honey had a significantly ($p<0.05$) higher FRAP value than the artificial honey and the total antioxidant activity of raw Millefiori honey was significantly different from processed Millefiori honey ($p< 0.001$). FRAP activity of the artificial honey was undetectable at the dilution used in the test, therefore indicating antioxidant compounds are responsible for FRAP values of honey samples rather than sugar content. Positive correlations were found between flavonoid content and the FRAP values (r^2=0.983) and between polyphenols and FRAP values (r^2=0.939). A linear relationship between total flavonoids content and total antioxidant power was also found.

The processing of honey often removes many of its phytonutrients (Wang, Gheldof & Engeseth, 2004 as cited by Blasa et al., 2006). Blasa et al. (2006) concluded unprocessed Millefiori honey can provide a source of dietary antioxidants but there is not enough evidence to assert honey is of primary importance in the human diet. However, its consumption can be recommended to complement other polyphenol sources in the diet such as vegetables and fruits (Blasa et al., 2006).

A study by Bertoncelj, Doberšek, Jamnik and Golob (2007) investigated the antioxidant properties of seventy samples from seven groups of honeys from various locations across Slovenia. They also investigated the phenolic content of the honeys using the Folin – Ciocalteu method as modified by Beretta, Granata, Ferrero, Orioli and Maffei Facino (2005). Bertoncelj et al. (2007) utilised the FRAP assay for the determination of the antioxidant capacity of the honeys and found significant differences ($p<0.05$) between the

honeys. The antioxidant activity of different honeys increased in the order: acacia< lime< multifloral<chestnut<spruce< forest< fir honey. Acacia honey had an average FRAP value of 71µmol/l, while the highest FRAP values were demonstrated by Slovenian fir and forest honey, 478.5 µmol/l and 426.4µmol/l respectively for the 10% solution. These results are similar to those obtained by Beretta et al. (2005) for chestnut and acacia honey; who found the least active honeys are those of monofloral origin (acacia, sulla, dandelion and clover). A positive linear correlation between the total antioxidant activity, determined by the FRAP method, and phenolic content was observed by Bertoncelj et al. (2007). This is consistent with the findings of other studies (Beretta et al., 2005; Blasa et al., 2006). Gheldof et al. (2002) as cited by Bertoncelj et al. (2007) suggested phenolic compounds significantly contribute to the antioxidant activity of honey. However, Bertoncelj et al. (2007) suggest that total antioxidant activity is due to the combined activity of honey phenolics, peptides, organic acids, enzymes and Maillard reaction products.

1.2.2.2 Antimicrobial activity of honey

Honey has been shown to have wound healing properties which can be ascribed to its antimicrobial activity. The antimicrobial activity can be effective against a broad spectrum of bacterial species especially those of medical importance (Basson and Grobler, 2008).

The antibacterial activity of honey has been investigated for its potential use in reducing food borne pathogens (Taormina, Niemira & Beuchat, 2001 as cited by Lusby et al., 2005); preventing catheter exit/entry infection (Quadri & Huraib, 1999 as cited by Lusby

et al., 2005); treatment of colitis (Bilsel, Bugra, Yamaner, Bulut, Cevikbas & Turkoglu, 2002 as cited by Lusby et al., 2005) and even protecting the gastric mucous in *H. pylori* induced inflammation (Ali, 2003; Osata, Reddy & Graham, 1999 both as cited by Lusby et al., 2005).

The inhibitory activity of honey has been attributed to several key properties including osmotic effect, low pH, production of hydrogen peroxide (Weston, Mitchell & Allen 1999) as well as the presence of phenolic acids, lysozyme and flavonoids (Cooper, Molan & Harding, 1999 as cited by Patton, Barnett, Brennan & Moran 2006) volatiles, beeswax, nectar, pollen and propolis (Weston, 2000).

White, Jubers and Schepatz (1963) as cited by Taormina, Niemira and Beuchat (2001) reported hydrogen peroxide, produced by glucose oxidase from the hypopharyngeal glands of honey bees, is the major antibacterial factor in honey. While the monosaccharides in honey are acquired from flower nectar, the enzymes originate from the glands of bees (White as cited by Weston et al., 1999). The addition of the enzyme catalase breaks down the hydrogen peroxide in an aqueous solution of honey; but residual non- peroxide antibacterial activity was still found in several honeys (Molan & Russell as cited by Weston et al., 1999).

Catalase, originating from pollen, can also be present in honey. According to Weston (2000), the level of hydrogen peroxide in a honey is determined by relative levels of glucose oxidase and catalase. A high glucose oxidase level corresponds to a high peroxide level whilst a low catalase level corresponds to a high peroxide level (Taormina

et al., 2001). These variations may account for the differences in antimicrobial activity among honeys from different floral sources.

Weston, Brocklebank and Lu (2000) found non peroxide factors may also contribute to antimicrobial properties of honeys. Phenolic antioxidants have been found to inhibit growth of a wide range of Gram negative and Gram positive bacteria (Davidson, 1993 as cited by Taormina et al., 2001). Pigments including carotenoids and flavonoids contribute to the dark colour of honeys and many have antioxidant activity. A positive correlation between antioxidant properties and both water content and colour of honey was found by Frankel et al (1998) as cited by Taormina et al., 2001).

Manuka honey is sourced from *Leptospermum scoparium*, a shrub native to the drier east coasts of the North and South Islands of New Zealand. Australian tea tree honey is sourced from *Leptospermum polygalifolium* which is predominantly found in Tasmania, Victoria and New South Wales. Tea tree and manuka honeys marketed as 'active' are reported to contain unique antibacterial properties determined by independent laboratories. In addition to hydrogen peroxide activity found in all honeys, tea tree and manuka honeys also exhibit non hydrogen peroxide activity. Non hydrogen peroxide activity or Unique Manuka Factor (UMF) is an indicator of the honey's antibacterial strength. Research has found that certain manuka honeys have been found to exhibit antimicrobial properties that cannot be due to the presence of hydrogen peroxide (Allen, Molan & Reid, 1991; Molan & Russell, 1988 both as cited by Adams, Boult, Deadman, Farr, Grainge, Manley-Harris et al., 2008). Weston, Mitchell and Allen (1999) concluded

phenolic products in manuka honey are only partly responsible for the observed non peroxide antibacterial properties of manuka honey. The non-peroxide antibacterial activity of manuka honeys is believed to be due to honey components derived from the floral source (Molan & Russell as cited by Weston et al., 1999). This deduction is supported by the observation that not all manuka honeys have non-peroxide antibacterial activity. Instead, the bioactivity was recorded in manuka honey from specific localities (Molan as cited by Weston et al., 1999); manuka honeys are graded using a unique manuka factor (UMF); the higher the UMF the more potent the antibacterial properties.

The potency of the non-peroxide antibacterial activity in 'active' manuka honey is measured using the agar well diffusion assay as described by Allen, Molan and Reid (1991). The enzyme catalase in added to the samples of honey to destroy all hydrogen peroxide in the honey. Therefore, only the non-peroxide antibacterial activity of the manuka honey is measured. The antibacterial activity of the honey is tested against *S. aureus* and compared with a standard antiseptic (phenol or carbolic acid) at various concentrations. The non-peroxide antibacterial activity of manuka honey is then reported as the equivalent concentration of phenol with the same level of antibacterial activity. For example, a honey with a UMF of ten will inhibit *S. aureus* as effectively as a 10% phenol solution.

UMFs range from 5+ to 30+, the UMF is marketed as a guarantee that the honey has been tested and verified for its antibacterial activity but does not measure a honeys antioxidant activity. Honeys with a UMF are more expensive, for example, a 250g tub of manuka

30+ costs approximately six times more than a 250g tub of manuka 5+ and eighteen times more than a 250 g jar of 'standard' clear honey, with no UMF,[1].

The disc diffusion assay is used routinely for testing common, rapidly growing and certain fastidious pathogens (Silici & Koc, 2006). Taormina et al. (2001) determined if six honeys from six different floral sources were lethal to, or inhibited the growth of, six food borne pathogens. The researchers also assessed the influence of the presence of hydrogen peroxide and level of antioxidant power against survival and growth of the pathogens, which included *E.coli* and *S.aureus*. The disc diffusion assay was used to assess honeys of different dilutions for inhibition of growth of pathogens. The study found inhibition was most evident around the discs soaked in the highest concentrations of solutions and *S.aureus* was most sensitive to the range of honeys examined. The authors found strains of five of the six pathogens did exhibit sensitivity to one of more honeys at concentrations of 25% or less. Three of the unprocessed honeys were more inhibitory than processed honeys to growth of five of the six food borne pathogens. The darker, opaque, unprocessed honeys were found to have the highest antioxidant potency when analysed using the FRAP assay. Taormina et al. (2001) concluded this correlation would support the theory that antioxidants present in honey contribute to antibacterial activity. Furthermore, Taormina et al. (2001) treated honeys with catalase to eliminate hydrogen peroxide. Growth of some strains, including *S. aureus,* in catalase treated solutions of unprocessed honeys were inhibited compared to growth in processed honeys. Taormina et al. (2001) found significant results ($p < 0.05$): the zone of inhibition of

[1] Price comparison calculated using prices of honeys purchased from Holland and Barrett in September 2008.

S.typhimunim H3402, surrounding discs soaked in a 25% solution of avocado honey, was significantly larger than the other honeys. Discs soaked in a 25% Chinaso buckwheat, blueberry, avocado or clove honey solutions produced significantly greater inhibition of *S. sonnei* compared with artificial honey. Growth of *S. sonnei* was significantly more inhibited by Chinaso buckwheat honey than 20% or 25% concentrations of all other honeys. Significantly larger zones of inhibition occurred against of *S.aureus* using discs soaked in blueberry or avocado honey

Taormina et al. (2001) found darker honeys were generally more inhibitory that light coloured honeys. However, no single honey exhibited exceptional inhibitory activity. The inhibition of growth of *S. sonnei, L.monocytogenes* and *S. aureus*, in 25% solutions of honey, was reduced by treating solutions with catalase; indicating hydrogen peroxide contributes to antimicrobial activity. Antimicrobial activity of darker coloured honeys was not eliminated by catalase treatments, suggesting non peroxide components such as antioxidants may contribute to controlling growth of some food borne pathogens. This finding suggests non peroxide components in honey do contribute to antibacterial activity but does not support the conclusion of Weston (2000) that non peroxide antibacterial activity reported in manuka honey should be interpreted as residual hydrogen peroxide activity. Taormina et al. (2001) concluded antibacterial properties of honeys containing hydrogen peroxide are characterised by antioxidant potency and need to be validated using modified systems.

Snow and Manley-Harris (2004) used the well diffusion assay to investigate the antimicrobial activity of an UMF 10+ manuka honey against *S. aureus*. The authors found the results for all five plates tested (32 wells per plate) overlapped indicating there

was little variation within and between plates. Snow and Manley- Harris (2004) added catalase to the manuka honey samples to test the hypothesis that non-peroxide activity is due to excess peroxide not destroyed by the catalase. Antimicrobial assays were performed using a 10 fold excess of catalase. Analysis of the resulting data showed there was no significant difference between using a standard volume of catalase and using an excessive volume. The authors concluded a standard volume of catalase was sufficient for the assay performed and that antimicrobial activity of the manuka honey tested manifested after the addition of catalase, showing it was non-peroxide activity.

There is evidence that honey with a high antibacterial activity could be used to reduce dental plaque in the treatment of oral disease (English, Pack & Molan, 2004 as cited by Basson & Grobler, 2008). Basson and Grobbler (2008) tested the antimicrobial activity of South African and manuka honeys against selected strains of oral micro-organisms using the broth dilution method. The aim of the study was to investigate the antimicrobial activity of two South African honeys and two honeys produced from alien species against selected reference cultures of potential oral pathogens including *S.mutans, E.coli* and *S. aureus*. Basson and Grobler (2008) observed no difference in the antimicrobial activity between the four honeys for concentrations up to 21%. At these concentrations, honey and the control solution had similar activity towards all organisms except two streptococcus species. No growth was observed at concentrations of 50% for the artificial honey control or for the honeys tested.

Basson and Grobler (2008) found the indigenous South African honeys did not display any exceptionally high broad spectrum antimicrobial activity. Furthermore, the manuka

honey did not significantly inhibit bacterial growth at lower concentrations. However, not all manuka honey exhibits antimicrobial activity (the study did not state if the manuka honey had a UMF). High antimicrobial activity is recorded only in manuka honey produced from specific localities in particular, the East Cape region of the North Island of New Zealand (Molan, 1995 as cited by Basson & Grobler, 2008).

Lee, Churrey and Worobo (2008) compared the antimicrobial activity of domestic honeys from the USA and two manuka honeys (UMFs not stated) against various bacterium including *S.aureus* and *E.coli*. When tested against two foodborne pathogen indicator strains, (*L.monocytogenes F2-586 1053* and *S. aureus ATCC 9144*), the six varieties of honey harvested in North America exerted relatively lower activity than the two manuka honeys from New Zealand. *L.monocytogenes* was found to be more sensitive than *S. aureus* to both manuka honeys tested.

Lusby, Coombes and Wilkinson (2005) studied the antibacterial activity of three locally produced honeys and compared them with three commercial therapeutic honeys including Medihoney® and manuka honey. The agar dilution method was used to assess the activity of honeys against 13 bacteria, including *E.coli*, *S. aureus* and *S. epidermidis* and one yeast. The honeys were tested at five concentrations ranging from 0.1 to 20%.

The authors found 12 of the 15 bacteria were inhibited by all honeys; *Serratia marcescens* and the yeast *Candida albicans* were not inhibited by the honeys. Little or no antibacterial activity was seen at honey concentrations below 1%, with minimal inhibition at 5% and no honey was able to produce complete inhibition of bacterial growth. Although Medihoney® and manuka honey had the overall best activity; the

locally produced honeys had equivalent inhibitory activity for some, but not all, bacteria. At higher concentrations there was a progressive increase in inhibition as honey concentration increased. Additionally, the manuka honey, Medihoney® and red stringy bark honey produced greater inhibition than the other honeys against *A.faecalis*, *E.aerogenes* and *S.aureus*.

The difference in minimum inhibitory concentration for antibacterial activity between UMF honeys and other honeys are often small (<5%) (Wilix et al., 1992; Molan and Bretl, 1998 all as cited by Lusby et al., 2005). Lusby et al. (2005) concluded honeys, other than those commercially available as antibacterial honeys, can have equivalent antibacterial activity and may prove to be a valuable source of future therapeutic honeys.

To date few studies compare the antioxidant activity of propolis with other bee products and with pure compounds. For example, the study by Watson et al., (2006) compared the ferric reducing capacity of samples of propolis from different geographical origins but not with honeys or other bee products. The nature of the antimicrobial activity of manuka honey has been extensively reviewed to date (Patton et al., 2006), but papers comparing manuka honey with a range of other bee products were also not found.

1.2 Aims:

The aim of this study was to evaluate the antioxidant and antimicrobial properties of bee products commercially available in the UK.

1.3 Objectives:

a) To investigate and compare the antimicrobial properties of bee products using the disc diffusion assay.
b) To investigate and compare the antioxidant properties of bee products using the FRAP assay.

1.4 Hypotheses:

1.4.1 Primary hypothesis:

- "Bee products demonstrate antibacterial and antioxidant properties".

1.4.2 Secondary hypotheses:

- "Antibacterial and antioxidant properties will differ between products".
- "Antibacterial properties of each product will differ between microbes tested."
- "Antibacterial and antioxidant properties will increase as the concentration of the products increase".
- "Antibacterial and antioxidant properties of manuka honey will increase as the unique manuka factor (UMF) increases".