Chapter Four: Case Study.

Evaluation of the antioxidant and antibacterial activity of human saliva before and after consumption of a propolis lozenge.

4.1 Introduction

The oral cavity is inhabited by up to one thousand different bacterial species at 10^8-10^9 per millilitre of saliva (Allaker & Douglas, 2009). Dental plaque is a film of microorganisms on the tooth surface that plays an important role in the development of caries and periodontal diseases (Marsh, 1992 as cited by Koo et al., 2000). *Streptococcus mutans* can colonize the tooth surface and initiate plaque formation. This occurs through the bacteria's ability to synthesise extracellular polysaccharides from sucrose using glucosyltransferase enzymes. (Gibbons & Von Houte, 1975; Freedman & Fitzgerald, 1985, as cited by Koo, Gomes, Rosalen, Ambrosano, Park & Cury, 2000). The development of pathogenic plaque is dependant on the sucrose adherence and accumulation cariogenic streptococci. Accumulation of plaque around the gingival margin and subgingival region may lead to a change in microbial composition from streptococcus to caprophilic and anaerobic bacteria such as *Porphyomonas gingivalis* (Marsh, 1994 as cited by Koo et al., 2000). Dental caries (dental decay) is a condition that results in the destruction of the dental hard tissues; the disease process involves acidogenic plaque bacteria such as *Streptococcus sobrinus*, *Lactobacillus spp.* and *Streptococcus mutans*. Dental caries can progress to inflammation and death of vital pulp tissue, spread to peripheral areas of the tooth and beyond (Allaker & Douglas, 2009). Colonization of the aforementioned bacteria can lead to root caries and periodontal

disease. Recent research has also suggested a link between oral microbionta and systemic disease such as cardiovascular disease (Beck & Offenbacher, 2005, as cited by Allaker & Douglas, 2009). Therefore, antimicrobial agents against oral pathogens could play an important role in the prevention of dental caries, periodontal disease and possibly systemic conditions.

Mechanical removal of plaque can help in preventing dental caries and periodontal diseases. Focus is now also directed towards the use of antimicrobial products derived from plants in preventing dental caries. According to Allaker and Douglas (2009), mainstream medicine is increasingly receptive to the use of antimicrobials derived from plant sources as traditional antimicrobial agents become ineffective against both existing and new microbial diseases.

Natural antibacterial substances are already being used as ingredients in mouth rinses and toothpastes, for example oils extracts of tea tree oil, peppermint, green tea and Manuka honey. Manuka honey has been tested for inhibitory activity against cariogenic and periodontopathic bacteria and been found to inhibit the growth of these bacteria. This may be due to the glucose oxidase it contains which generates hydrogen peroxide in the presence of water. Manuka was also found to inhibit the adhesion of *Streptococcus mutans* (Takarada, Kimizuka, Takahashi, Honma, Okuka & Kato, 2004, as cited by Allaker & Douglas, 2009).

Propolis has attracted increased interest due to its antimicrobial activity against a wide range of pathogenic microorganisms. Antimicrobial activity against *Streptococcus*

mutans has been previously reported by several researchers (Ikeno, Ikeno & Miya, 1991; Steinburgh, Kain & Gedalia, 1996; Park, Koo, Abreu, Ikegaki, Cury & Rosalen, 1998 all as cited by Koo et al., 2000). Park, et al. (1998) (as cited by Bosio et al. 2000) found ethanolic extracts of propolis inhibited both the growth of *Streptococcus mutans* and its production of glucosyltransferase, the enzyme responsible for the formation of dental plaque and caries. Propolis can now be found in mouthwashes and toothpastes to help prevent caries and treat gingivitis and stomatitis (Pietta et al, 2002).

A case study was conducted to investigate the antibacterial and antioxidant properties of saliva after a propolis lozenge had been consumed.

Hypotheses:

- The FRAP value of saliva will be increase after the consumption of a propolis lozenge.
- The viable count of oral bacteria will be reduced after the consumption of the propolis lozenge.

The study involved one subject, the researcher; therefore ethical approval was not needed.

4.2 Methods

The disc diffusion assay and the FRAP assay were used, as described in chapter two.

4.2.1 Materials

The samples evaluated were:

- Human saliva,
- Saliva after consuming a generic boiled sweet (control) (Werther's Original®, purchased from Tesco Extra, Winwick Road, Warrington).
- Saliva after consuming a propolis lozenge (brand Bee Health, purchased from Holland and Barrett, Golden Square, Warrington).

4.2.2 Procedures

The samples were obtained first thing in the morning (before breakfast and before teeth were brushed since the previous night). Samples were obtained during the consumption of the lozenges. The mouth was rinsed with tap water between consumption of the boiled sweet and the propolis lozenge. The samples were obtained daily and stored in sterile universal bottles.

Firstly, samples of saliva, saliva and boiled sweet and saliva and propolis lozenge were used, undiluted, in the disc diffusion assay to determine antibacterial properties against the bacterium (*E.coli, S. aureus, S.epidermidis* and *S. mutans*).

Samples were also analysed in triplicate using the FRAP assay to determine the antioxidant activity of saliva, saliva and boiled sweet and saliva and propolis lozenge.

The samples (dilutions 10^{10} to 10^{7}) were also spread, in triplicate, on nutrient and glucose broth agar to determine if propolis had an affect on the viable count of bacteria in the mouth immediately after (time zero) and five minutes after consumption of the propolis lozenge. Viable counts were taken 24 hours after the samples were spread on

agar plates and 72 hours after the samples were spread on agar plates. Samples were diluted with sterile water.

4.3 Results

4.3.1 Antioxidant activity (FRAP)

Table 9. Antioxidant activity of saliva (FRAP).

Product	Dilution (%)	Mean FRAP value (µmol/l)
Saliva	Undiluted	630 ± 200
Saliva	50	383 ± 20
Saliva	25	347 ± 5
Saliva	12.5	293 ± 11
Saliva	6.25	250 ± 43
Saliva	3.125	157 ± 57

Table 10. Antioxidant activity of saliva with boiled sweet (FRAP).

Product	Dilution (%)	Mean FRAP value (µmol/l)
Saliva + Boiled Sweet	Undiluted	240 ± 96
Saliva + Boiled Sweet	50	180 ± 10
Saliva + Boiled Sweet	25	82 ± 23
Saliva + Boiled Sweet	12.5	125 ± 32
Saliva + Boiled Sweet	6.25	45 ± 49
Saliva + Boiled Sweet	3.125	0 ± 0

Table 11. Antioxidant activity of saliva with propolis lozenge (FRAP).

Product	Dilution (%)	Mean FRAP value (µmol/l)
Saliva + Propolis Lozenge	Undiluted	603 ± 85
Saliva + Propolis Lozenge	50	343 ± 66
Saliva + Propolis Lozenge	25	158 ± 7
Saliva + Propolis Lozenge	12.5	85 ± 0
Saliva + Propolis Lozenge	6.25	27 ± 2
Saliva + Propolis Lozenge	3.125	13 ± 10

4.3.2 Disc Diffusion

Saliva and saliva after consumption of the boiled sweet failed to inhibit the growth of any of the bacterium. Saliva after the consumption of the propolis lozenge failed to inhibit the growth of *E.coli, S. aureus,* and *S. mutans.* Inhibition was detected against *S.epidermidis*; (mean zone of inhibition 2.6mm).

4.3.3 Viable Count

Table 12. Mean viable count at 24 hours and 72 hours.

Product	Dilution	Mean viable count 24 hours	Mean viable count 72 hours
Saliva	10^{-7}	36 ± 8	83 ± 9
Saliva + boiled sweet (0 mins)	10^{-7}	48 ± 2	101 ± 7
Saliva + boiled sweet (5 mins)	10^{-7}	41 ± 16	109 ± 29
Saliva + propolis lozenge (0 mins)	10^{-7}	73 ± 9	156 ± 17
Saliva + propolis lozenge (5 mins)	10^{-7}	47 ± 16	94 ± 31

4.4 Discussion and Conclusion

Tables 9, 10 and 11 show dose response between antioxidant activity (FRAP value) and dilution of test substances. The antioxidant activity of the saliva plus propolis lozenge is higher than that of saliva plus boiled sweet. The antioxidant activity of saliva on its own is the highest of the samples. This may be due to increased volume of saliva, and therefore antioxidant compounds becoming diluted during consumption of the sweet and lozenge.

Table 12 shows viable counts increased for all samples between 24 hours and 72 hours. The samples collected five minutes after consumption of the boiled sweet and the propolis lozenge produced lower viable counts. However, no conclusions can be made from the data as the exact composition of the sample saliva was not investigated and therefore the effects of indigenous flora and oral enzymes such as amylase on the results are not known.

Further study is needed to be able to generalise the results to a wider population as these results are only applicable to the participant in the study.