# PLANT-DERIVED BIOCIDES FOR WATER DISINFECTION AND OTHER HYGIENIC APPLICATIONS

By

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**Statement of own work**

The contents of this thesis are all my own work, and any quotation from, or description of, the work of others is fully acknowledged by reference to the sources, whether published or unpublished.

**Abstract**

The production of potentially harmful disinfection by-products and the emergence of resistant bacteria have generated interest in searching for new antimicrobial agents that are safe and ideally similarly cheap compared to existing biocides. This research aimed to investigate the inhibitory activity of extracts and individual compounds derived from banana peel, a major food waste in many parts of the world, on bacteria (*Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 12241) and viruses (represented by a viral surrogate, MS2 coliphage). To better understand the antimicrobial activity, generation of hydrogen peroxide, one of the potential antimicrobial mechanisms involved, was also studied.

In the first part of this research, the relationship between extract composition and antimicrobial activity was studied. Banana peels subjected to one of two drying processes, freeze drying and oven drying, were extracted at different maceration times and the antimicrobial activity of the extracts was determined using a broth micro-dilution method. Freeze-dried banana peels extracts from three hours of maceration were found to be the most active for both bacteria tested. Following this, compound identification was carried out on the freeze-dried banana extracts using high-performance liquid chromatography (HPLC) to determine the active compounds that are responsible for the extracts' antimicrobial activity. Three compounds, namely gallic acid, catechin and epicatechin, were found to be present in all extracts and therefore were chosen for further study individually. Epigallocatechin gallate (EGCg), a related catechin that has already been studied by others previously, was also included.

To determine the relative efficacy of the extracts and their individual compounds as biocides, a disinfection experiment was carried out. The results showed that higher inhibitory activity by the extracts was observed in *S. aureus* than *E. coli*. Among all individual compounds tested, EGCg was shown to have the most potential to be explored as a biocide, meanwhile only relatively weak activity (i.e. less than 0.5-log reduction) was exhibited by the banana peel-derived compounds. Nevertheless, the combination of two banana peel-derived antimicrobial compounds was shown to improve the inhibitory activity – i.e. higher *S. aureus* reduction was achieved compared to the sum of the reduction achieved when the compounds were tested individually, though no synergy between the compounds was observed for *E. coli*.

Using a plaque assay, possible antiviral activity of the extracts and individual compounds against MS2 coliphage was determined. The results showed that MS2 was highly resistant to the extracts and all compounds tested, suggesting that banana peel extracts and their individual compounds may not be suitable to be used as virucides.

Previously the inhibitory activity of these compounds had been suggested to be potentially due to the generation of hydrogen peroxide. Therefore, in this study the amounts of hydrogen peroxide generated were quantified using the ferrous oxidation-xylenol orange (FOX) assay. The results showed that micro-molar concentrations of hydrogen peroxide were indeed generated under the conditions of the disinfection experiments. This value however is too low for significant reduction in bacterial viability to be observed, and instead it was likely that the inhibitory action was mainly due to other mechanisms, such as binding to vital components in the cells. The results also indicate that the inhibitory action of EGCg treatment was not due to hydrogen peroxide generation, but likely due to direct interaction of the molecule to the peptidoglycan with cells, as proposed by other researchers.

Overall, this study was the first to evaluate the efficacy of natural biocides derived directly from a waste material, against a range of target microorganisms. The results of this study demonstrate the potential of some banana peel-derived individual compounds against some species of bacteria, and demonstrated that there are synergies between two of the compounds in particular. Potential future commercialisation of such compounds as biocides (e.g. EGCg shows the most promise) would require further optimisation of these synergies and a method for cost-effectively scaling up the extraction procedures that are presented in this thesis.

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**Glossary**

| | |
|---|---|
| AITC | Allyl isothiocyanate |
| ATCC | American Type Culture Collection |
| C | Catechin |
| cfu | Colony forming unit |
| CHX | Chlorohexidine |
| CLSI | Clinical and Laboratory Standard Institute |
| CRA | Chlorine releasing agent |
| DBPs | Disinfection by-products |
| DMSO | Dimethyl sulphoxide |
| DNA | Deoxyribonucleic acid |
| DW | Dry weight |
| DWI | Drinking Water Inspectorate |
| EC | Epicatechin |
| ECg | Epicatechin gallate |
| EC50 | Half maximal effective concentration of drugs |
| EDTA | Ethylenediaminetetraacetic acid |
| ECg | Epicatechin gallate |
| EGCg | Epigallocatechin gallate |
| EGC | Epigallocatechin |
| EMEA | European Medicine Evaluation Agency |
| EOs | Essential oils |
| FAO | Food and Agriculture Organization |
| FCV | Feline Calicivirus |
| FDA | Food and Drug Administration |
| FIC | Fractional inhibitory concentration |
| FOX | Ferrous Orange Xylenol |
| GC | Gallocatechin |
| GCg | Gallocatechin gallate |
| GRAS | Generally Recognised As Safe |
| $H_2O_2$ | Hydrogen peroxide |

| | |
|---|---|
| HCV | Hepatitis C |
| HIV | Human immunodeficiency virus |
| HOCl | Hypochlorous acid |
| HPC | Heterotrophic plate count |
| HPLC | High Performance Liquid Chromatography |
| HSV | Herpes Simplex Virus |
| LPS | Lipopolysaccharide |
| M | Unit for molar |
| mM | Unit for milimolar |
| MIC | Minimum inhibitory concentration |
| MHB | Mueller Hinton Broth |
| MNV | Murine norovirus |
| MTT | Name for colorimetric assay used for cell metabolic activity assessment |
| MS2 | Male specific bacteriophage |
| MRSA | Methicillin Resistant Staphylococcus aureus |
| N | Normality |
| NaOH | Sodium Hydroxide |
| NaCl | Sodium Chloride |
| NCTC | National collection type culture |
| NTU | Unit for turbidity |
| OD | Optical density |
| -OH | Hydroxyl group |
| PAC | Proanthocyanidins |
| PBS | Phosphate buffer solution |
| pfu | Plaque forming unit |
| PHE | Public Health England |
| pH | Logarithm (base 10) of the hydrogen ion concentration |
| pI | Isoelectric point |
| pKa | Symbol used for acid dissociation constant at logarithmic scale |
| QACs | Quaternary ammonium compounds |
| recA | Name of protein responsible for DNA repair |
| RNA | Ribonucleic acid |

| | |
|---|---|
| RO | Reverse osmosis |
| rpm | Rotation per minute |
| RSV | Respiratory syncytial virus |
| SFE | Supercritical Fluid Extraction |
| SOD | Superoxide dismutase |
| TEM | Transmission electron microscopy |
| TSA | Tryptone soya agar |
| TSB | Tryptone soya broth |
| UV | Ultraviolet |
| WHO | World Health Organisation |

# Chapter 1 INTRODUCTION

## 1.1 Research Motivation

A 'biocide' is any substance used to prevent or control the growth of unwanted microorganisms through chemical or biological action. The term 'biocides' is also sometimes used when referring to antiseptics, pesticides and disinfectants. For the purpose of this study, the term biocide will be used to refer to its application as a **disinfectant**. Early applications of biocides include the use of salts and spices as preservatives, wines as wound cleansers and silver for water storage (Myers, 2008). However, since the industrial revolution, a broad range of chemical agents have been discovered (**Table 1-1**) and used in many biocide products, including the use of chlorinated agents in water treatment, quaternary ammonium compounds (QACs) in cleaning products and also the use of acids and bases. There is no doubt that since their introduction, the application of chemical biocides has become an essential part of providing the public with a high level of protection from the transmission of pathogenic diseases, and also preventing microbial-induced damage to various household products and environments. This is evidenced through the increased demand for biocides in the global market, which was valued at 6 million USD in 2015, and is projected to increase to up to 9 million USD in 2022, making the biocide industry one of the fastest growing industries in the world (Satsangi, 2017).

Despite all of their benefits and the high demand for biocides, there is growing concern among the public that the widespread use of these synthetic biocides may have adverse effects on human health and the environment. Owing to the toxic nature of most biocide compounds, some researchers also believe that the increased number of cases of health and skin problems, particularly among newborn babies, may be linked to the overuse of chemical biocides (Levy, 2001). Moreover, the application of disinfectants such as chlorine, chloramine and ozone in water disinfection treatment has also been shown to contribute significantly to the production of disinfection by-products (DBPs). While ongoing research on DBPs is being carried out, previous epidemiological studies have suggested that the long-term consumption of chlorinated water is

associated with occurrence of bladder cancer in both men and women (Villanueva et al., 2003). Currently, more than 600 DBPs have been identified and various studies suggest their potential health risks to humans and the environment (Richardson, 2011). More importantly, it is also feared that the application of these chemical biocides may eventually lead to the emergence of resistance among bacteria. Indeed, studies have reported a reduced susceptibility of bacteria to antibiotics after exposure to QAC products (Russell et al., 1998).

Therefore, there is an urgent need among researchers to look for an alternative to chemical biocides, and attention has been given to plants as a source of antimicrobial agents. For many centuries, plants have been used traditionally for the treatment of common illness, including infection. Plants in general have a limitless ability to synthesise different kinds of molecular substances that are responsible for their defence mechanism against stress – infections and environmental stresses that they encounter in their lives (Cowan, 1999; Abreu et al., 2012; Daglia, 2012). It is estimated that around 500,000 plant species are available on Earth, although only 10% of them have been tested for biological activities (Cowan, 1999). A great deal of research has been conducted on plant biological activities, and much of this has suggested that plants have a therapeutic potential on human health and for combating microbial infections. In fact, around 25% of clinically used drugs are reported to be synthesised from higher plant-derived products (Phillipson, 2007; Abreu et al., 2012). In spite of this, there are still a limited number of studies being carried out to determine whether plant-derived compounds, either as extracts or purified compounds, can be used as biocides for disinfection.

While most previous studies on plant antimicrobial activity have focused on determining the inhibitory concentration of plant-derived compounds, attempts have also been made by researchers to use plants compounds as biocides for water disinfection. For instance, the application of plant extracts (Neem oil) for inhibiting waterborne coliform in surface water has suggested that natural biocides have potential for further exploration (Matthews et al., 2009). Using pure natural compounds such as allyl isothiocyanate (AITC), studies also shown that individual plant-derived compounds are able to reduce the growth of *E. coli* and natural bacterial populations (Mushantaf et al., 2012). These studies have therefore highlighted plant-derived

compounds either in mixed (extract form) or isolated compounds, that could be used as microbial growth inhibitors or biocides.

***Table 1-1**: Introduction year of chemical biocides.* Adapted from Russell (2002)

| **Biocides** | **Year discovery/ first description** | **Introduction/ application** |
|---|---|---|
| **Alcohols** | BC | Early AD |
| **Chlorine** | 1774 | 1847 |
| i. **Sodium hypochlorite** | 1789 | 1827 |
| ii. **Chlorine Releasing Agents (CRA)** | 1915 | 1916 |
| iii. **Chlorine dioxide** | 1925 | 1946 |
| **Iodine** | 1812 | 1816 |
| i. **Iodophores** | 1949 | 1956 |
| **Formaldehyde** | 1867 | 1894 |
| **Phenols** | | |
| i. **Phenol** | 1834 | 1867 |
| ii. **Cresol** | 1842 | 1890 |
| iii. **Chlorocresol** | 1906 | 1908 |
| iv. **Bisphenols** | 1906 | 1927 |
| v. **Triclosan** | 1906 | Early 1970s |
| **Quaternary ammonium compounds (QACs)** | 1856/1916 | NA |
| **Chlorhexidine** | 1946 | NA |
| **Amphoterics** | 1952 | NA |
| **Peracetic acid** | NA | 1955 |
| **Glutaraldehyde** | 1904 | 1960s |

NA-not available

Nevertheless, it should be noted that previous disinfection studies using natural biocides have mainly focused on the application of essential oils (EOs) as the biocidal compounds. Essential oils, although effective in inhibiting broad spectrum bacterial species (Burt, 2004), have a limitation for water application studies, due to their hydrophobic nature, i.e. they do not mix into water. Some researchers argue that their inhibitory mechanisms are due to the formation of two immiscible phases after EOs are added to water (Hili et al., 1997; Friedman et al., 2002). Naturally derived antimicrobial compounds in general can be found in any parts of plant components, including their by-products. Several reports on plant by-products have shown that by-products of fruits and vegetables, in particular, are a rich source of plant secondary metabolites such as tannins, terpenoids, alkaloids, and phenolics (Balasundram et al., 2006). Some of these plants components have been tested for their antimicrobial activity and shown to be active antimicrobials.

Banana is an important crop for food security and also a large source of food waste globally. Being grown mostly in developing countries, bananas are known for their nutritional value and benefit to human health. Nevertheless, banana processing often produces a significant amount of waste by-products such as the peels. Banana peels account for 30% of the fruit's weight, and are a rich source of plant compounds (phytochemicals); they have been studied previously for their antimicrobial activity. Traditionally, banana peel has been used for centuries in Brazilian medicine for the treatment of wounds, as the inside part of the peel is known to be antiseptic (Pereira and Maraschin, 2015). The high inhibitory activity of the ethyl acetate extracts of green banana peels against five different bacteria species – *Bacillus cereus*, *Salmonella enteritidis*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* – have also been reported previously (Mokbel and Hashinaga, 2005). Taking this into consideration, this study therefore aimed to discover the antimicrobial activity of this abundant natural material as a biocide, so that it may be considered as a disinfectant in water disinfection or hygienic applications.

## 1.2 Research objectives

The overall aims of this study were to evaluate the potential of banana peel extracts as a biocide solution, for use in either water disinfection or other hygienic applications (e.g. in household cleaning products).

The specific scientific objectives of this study were:

- To establish the phenolic profile of banana peel extracts obtained through optimised conditions that lead to extracting solutions with maximum antimicrobial action.
- To determine the ability of banana peel extracts in mixed and individual compound forms to inhibit the growth of bacteria, and understand whether the extract's inhibitory activity is related to individual compound activity.
- To find the best combination of individual polyphenol compounds, with different mechanisms of action, that may work in synergy in inhibiting *bacterial* growth.
- To determine whether the obtained biocides may also have capabilities for inactivating viruses.
- To determine the effects of the hydrogen peroxide generated from the extracts/individual compounds on the observed antimicrobial activity.

## 1.3 Thesis outline

Chapter 1

This chapter explains the problems associated with current biocides, summarising previous studies that have been conducted in relation to the topic, to fully emphasise the gaps in knowledge. It also sets out the aim and objectives of the research.

Chapter 2

This chapter provides an introduction to biocides and classes of plants-derived chemical compounds, with a focus given especially to the compounds examined in this study. Previous studies on natural biocides in water disinfection and banana peel antimicrobial activity are also

summarised. To allow a better understanding of the antimicrobial assessment methods discussed in the subsequent chapters, an introduction to the assessment methods is also provided.

Chapter 3

This chapter provides the details of the materials and methods that were used to generate the results that are discussed in the subsequent chapter.

Chapter 4

This chapter presents the results from all of the experiments conducted in the study. The results presented are divided into five main sections, in line with the objectives of the research: (i) extraction and compound identification, (ii) antimicrobial study of the extracts and compounds against *S. aureus* and gram negative bacteria, (iii) the antimicrobial synergy between the compounds, (iii) the antiviral study assessment and (iv) the relationship between hydrogen peroxide generation and inhibitory activity.

The data on EGCg disinfection as presented in this chapter was presented at 19th IWA-UK Young Water Professionals Conference, 16-19 April 2018, Cranfield University.

Chapter 5

This chapter presents an overall discussion, drawing together the various experimental findings from the research, and considers the application of these compounds as future biocides. Recommendations for future work are also proposed.

Chapter 6

This chapter highlights the main findings and original contributions to knowledge of the study.

Two journal publications are in preparation for submission in early 2019 entitled:

- Antimicrobial activity of banana peel extracts and their individual constituents towards *S. aureus*, *E. coli* and MS2 coliphage (target journal: *Environmental Technology*).
- Antimicrobial inhibitory synergies of polyphenols compounds derived from fruit waste extracts (target journal: *Applied and Environmental microbiology*).

Chapter 2 **LITERATURE REVIEW**

## 2.1 Introduction to biocides

The term 'biocide' can refer to any compound(s) that is used to kill or inhibit the growth of microbial species. In order to fully understand how biocides work, it is important to consider the main components involved in bacteria-biocide interactions. Unlike antibiotics, which attack specific sites on cell membranes, biocides, on the other hand, work on multiple or unspecified target sites (Seier-Petersen, 2013). In theory, biocides' target sites can be divided into three main regions: the cell wall, cytoplasmic membrane and cytoplasm. Each target site plays an important role in the cell's metabolism processes, as described in **Figure 2-1**.

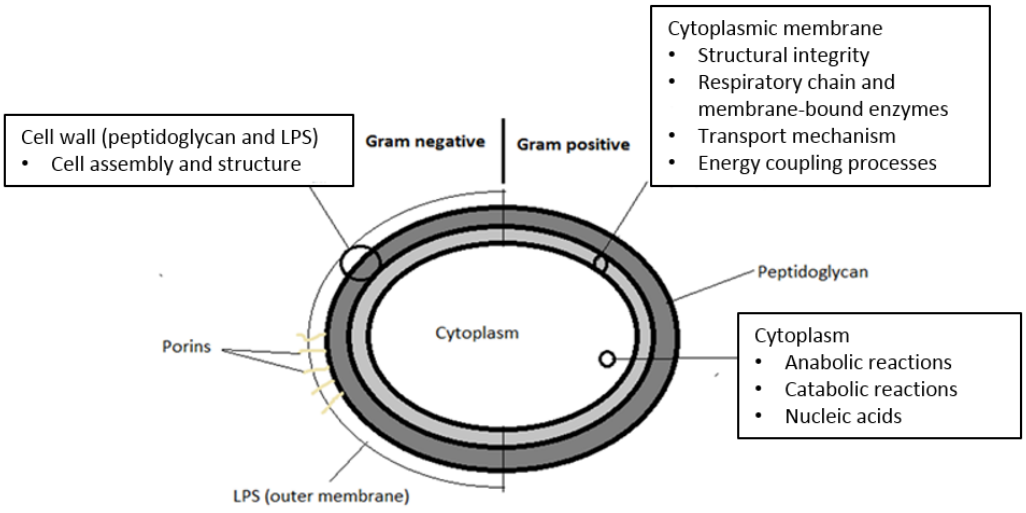



***Figure 2-1:*** *Illustrative diagram for Gram-negative and Gram-positive bacteria cell membranes* Adapted from Denyer and Stewart (1998)

The access of biocides to these three main regions will therefore determine the efficacy and the mode of action of the biocide agents. Nonetheless, it is generally accepted that the structure and composition of the outer cell wall play an important role in defining the susceptibility of microbial species to biocide agents (Denyer and Stewart, 1998). For example, Gram-negative bacteria and mycobacteria are recognised to have higher resistance to many biocide agents than Gram-positive bacteria, mainly due to the permeability barrier made by their

outer cell wall, which acts as an intrinsic resistance to biocide activity. Gram positive bacteria in general are defined as a group of bacteria that give a purple color when stained with crystal violet due to the presence of a thicker layer of peptidoglycan in their cells. Gram negative bacteria are characterised by a group of bacteria with outer membrane structure known as lipopolysaccharide (LPS) that covers the peptidoglycan cells, which gives negative results during staining (Madigan et al., 2000). Nevertheless, some agents such as ethylenediamine tetraacetic acid (EDTA) and glutaraldehyde known as permeabilisers, have been reported to exhibit their antimicrobial activity through interaction with the outer membrane structure (Denyer, 1995). Of all the target sites, the most cited target region for many biocide agents is known to be the cytoplasmic membrane (McDonnell and Russell, 1999). This is due to its large surface area, which is the main site for many fundamental cell metabolism processes such as enzymatic action, and also for maintaining cell structure integrity. Therefore any damage in this cytoplasmic region contributes to the release of intercellular components, and subsequently to cell death. In general, the susceptibility of organisms to biocide activity follows the order shown in **Figure 2-2**. Although this hierarchy may not be applicable to all biocide agents, but in most cases, this order is true.

The interaction of biocides with their target sites is hardly instantaneous, but often requires a certain degree of partitioning of biocide dose, followed by its accumulation at the target sites until a damaging level is reached. The resulting cells injury due to the biocide's action can be divided into two categories: bactericidal and bacteriostatic action. The former activity is normally achieved at a higher concentration of biocide dose, above its sub-lethal concentrations, which leads to permanent damage of the vital cellular components that are responsible for cell lysis (Denyer, 1995). Meanwhile, bacteriostatic action occurs at low biocide concentrations, and often causes a reversible metabolic injury to cell membranes and a reduction in cell viability (Denyer, 1995). However, the bactericidal effect is often harder to visualise as it requires detailed examination tools before it can be determined. Therefore in most cases, the antimicrobial activity of biocide agents is determined by looking at the bacteriostatic effects. The bacteriostatic activity

of biocides can be evaluated by measuring the minimum inhibitory concentration (MIC) required to inhibit the bacterial growth, and also through visualisation of the reduction in cell population.

| Susceptibility | | Microorganisms | Examples |
|---|---|---|---|
| Low |  | Prions | CJD, BSE, Scraple |
| |  | Bacterial spores | Geobacillus, Clostridium |
| |  | Protozoal oocysts | Cyptosporidium |
| |  | Helminth eggs | Ascaris, Enterobius |
| |  | Mycobacteria | Mycobacterium tuberculosis |
| |  | Small non-enveloped viruses | Poliovirus, Parvovirus |
| |  | Protozoal cysts | Giardia, Acanthamoeba |
| |  | Fungal spores | Aspergillus, Penicillium |
| |  | Gram-negative bacteria | Pseudomonas, Escherichia |
| |  | Vegetative fungi and algae | Aspergillus, Candida |
| |  | Vegetative helminths and protozoa | Ascaris, Cryptospordium |
| |  | Large non-enveloped viruses | Adenovirus, Rotavirus |
| |  | Gram-positive bacteria | Staphylococcus, Streptococus, Enterococcus |
| High |  | Enveloped viruses | HIV, HBV, HSV |

***Figure 2-2**: General order of organism susceptibility to biocides*

Adapted from McDonnell and Russell (1999) and Sakudo et al. (2010).

### 2.1.1 Factors affecting biocidal activity

Apart from variations in cell structure and compositions, several other factors are known to influence the efficacy of biocide activity. These include:

(1) Concentration

Concentration is the major factor in the study of biocide agents, and therefore the efficacy of biocides is often represented by the term 'minimum inhibitory concentration (MIC)'. It should be noted that most biocidal products are normally formulated at a concentration beyond their inhibitory concentration with the aim of ensuring a higher degree of elimination of various organisms. However, a factor such as dilution is known to influence the biocides' concentrations and activity. The effect of dilution on biocides concentration is often expressed by using a mathematical term called a concentration exponent (η). Concentration exponents can be determined by using the following equation 2.1 as described by Hugo and Denyer (1987):

$$\eta = \frac{(Log\ death\ time\ at\ concentration\ C_2) - (Log\ death\ time\ at\ concentration\ C_1)}{Log\ C_1 - C_2} \qquad \textit{Equation 2-1}$$

The concentration exponent reflects the time needed to kill the bacterial growth at particular concentration. It is generally accepted that the more concentrated the biocide agent, the less time is required to inhibit the microbial growth. Biocides with high concentration exponent (η) values, such as alcohols and phenolic compounds, which react through weak interaction (accumulation) to lipid components in the cell envelope, are recognised to be affected significantly by changes in concentrations and dilutions (Russell and McDonnell, 2000). Meanwhile, biocides with low η values, such as organomercurials, silver compounds, oxidising agents (peroxide, iodine), membrane-active agents ((chlorhexidine salts (CHX), quaternary ammonium compounds (QACs)), DNA reactors (acridines) and amino group reactors (formaldehyde), which interact with their bacterial sites through chemical or ionic bonding, are shown to be less affected by concentration gradients (Russell and McDonnell, 2000).

(2) Time

Contact time can be defined as the exposure time between the biocides and bacterial cells. In general, the duration of the antimicrobial agents applied can be classified in accordance with their application. For instance, at least 1 minute of contact time is required for testing biocides meant for hand disinfection purposes, and at least 5 minutes for general chemical disinfectants and antiseptics (British Standard, 2010). In the UK water sector, at least 30 minutes contact time is required for chlorination in drinking water systems (DWI, 2015).

(3) Biocide formulation

The addition of more than one antimicrobial agent into a biocide formulation can improve the efficacy of biocide agents. The combined treatment of two biocides with different modes of action has been shown to significantly improve the inhibitory activity. For instance, hydrogen peroxide has been reported to exhibit a temporary inhibition on *E. coli* growth at a low concentration, with increased cell viability observed at longer contact times (Brandi et al., 1989). Nevertheless, the combination of peracetic acid and hydrogen peroxide has been shown to act in synergy and dramatically improve the bactericidal activity of the compounds alone. In fact, several products exploiting this combination have been approved by the U.S. Food and Drug Administration to be used as sterilants and/or high-level disinfectants (Leggett et al., 2015).

(4) Extracellular condition

The presence of organic or inorganic constituents and the pH of the cell medium are known to interfere with biocides' activity. For instance, it is widely reported that highly turbid water generally has a higher resistance to biocide activity compared to pre-treated or filtered water influents. This is primarily due to the shielding effects provided by the attached particles, which prevent effective contact between the biocides and their target. Moreover, the presence of extracellular materials, particularly organic matter, could attribute to microbial aggregation, which increases bacterial resistance to biocide activity. Studies have also shown that pH could influence the uptake of biocides into cells' intracellular components. For instance, the inactivation of *E. coli* K-12 using monochloramine was shown to be four times faster at pH 7 than at pH 8 (Ward et al., 1984). Oxidising biocides, such as chlorine and monochloramine, are

known to exhibit their antimicrobial activity more effectively in their undissociated form, hypochlorous acid (HOCl), rather than $OCl^-$. At pHs between 4 and 7, the uncharged moiety, HOCl, can diffuse through the cell membranes better than negatively charged hypochlorite ions, $OCl^-$ which predominate at pHs above 7 (Chlorine Chemistry Council, 2003). In general, most weak acids are effective at a pH below their pKa; surfactants are often used at pHs that cause negatively charged surfaces on cellular membranes (Denyer and Stewart, 1998).

## 2.2 Plants as antimicrobial/antiviral sources

The application of plants as biological agents can be traced back to early human history; even during the time of Hippocrates, around 400 different plant species were utilised for medicinal purposes (Cowan, 1999; Cushnie and Lamb, 2005; Sarker and Nahar, 2009). Since the discovery of their therapeutic properties, plants have continued to be the main sources of medicines in many traditional medicines such as Ayurveda and Chinese herbal medicine (Sarker and Nahar, 2009). Indeed, as reported by the World Health Organization (WHO), around 75% of populations in many underdeveloped and developing countries still rely on plant-based treatment, indicating the importance of plants as the basic treatment for bacterial infections. Furthermore, approximately 60–80% of antibacterial and anticancer drugs have also been derived from natural origins, including plants such as vincristine from *Vinca rosea*, morphine from *P. somniferum*, and Taxol® from *T. brevifolia* (Sarker and Nahar, 2009).

It is estimated that 250,000 to 500,000 plant species are present on Earth, however only 1-10% of plants compounds has been discovered so far (Cowan, 1999). With advances in analytical tools, several plant compounds have been isolated, characterised and tested for their biological activities as antioxidant, antifungal, anticancer, antiviral and also antimicrobial agents. For the purpose of this thesis, only their application as antiviral and/or antimicrobial agents will be focused on.

It is well known that plants have a limitless ability to synthesise different chemical compounds, known as their secondary metabolites. These secondary metabolite compounds are produced as a result of pathogen invasion and environmental stress that plants experience in their

daily lives. The secondary metabolites of plants can be divided to several classes, such as polyphenols, terpenes, alkaloids, and tannins, but the largest proportion of secondary metabolites consists of phenols or their oxygen-substituted derivatives in their structure (Cowan, 1999), which are often referred to as polyphenols or phenolic compounds.

### 2.2.1 Phenolic compounds

Polyphenol/ phenolic compounds are the largest group of plant secondary metabolites, and they can be divided into several classes as shown in **Table 2-1**. Among all these classes, flavonoids, phenolic acids and tannins are considered to be the main polyphenol compounds that can be found in the human diet (Balasundram et al., 2006). Nevertheless, it should be emphasised that the selected compounds involved in this study mainly consisted of compounds from flavonoids and phenolic acid groups, therefore focus will be given to these two groups.

***Table 2-1**: Classes of plant-derived phenolic compounds*

(Adapted from Balasundram et al.(2006))

| **Class** | **Compounds configuration*** |
|---|---|
| **Simple phenolics, benzoquinones** | $C_6$ |
| **Hydroxybenzoic acids** | $C_6$-$C_1$ |
| **Acethophenones, phenylacetic acids** | $C_6$-$C_2$ |
| **Hydroxycinnamic acids, phenylpropanoids (coumarins, isocoumarins, chromones, chromenes)** | $C_6$-$C_3$ |
| **Napthoquinones** | $C_6$-$C_4$ |
| **Xanthones** | $C_6$-$C_1$-$C_6$ |
| **Stilbenes, anthraquinones** | $C_6$-$C_2$-$C_6$ |
| **Flavanoids, isoflavanoids** | $C_6$-$C_3$-$C_6$ |
| **Lignans, neolignans** | $(C_6$-$C_3)_2$ |
| **Biflavanoids** | $(C_6$-$C_3$-$C_6)_2$ |
| **Lignins** | $(C_6$-$C_3)_n$ |
| **Condensed tannins (proanthocyanidins or flavolans** | $(C_6$-$C_3$-$C_6)_n$ |
| ***C6 is the aromatic benzene ring** | |

#### *2.2.1.1 Flavonoids*

Flavonoids (**Figure 2-3**) are the largest group of plant secondary metabolites, and can be found in a wide variety of fruits and vegetables consumed by humans. In general, about 4000 flavonoids have been identified (Daglia, 2012)), and these flavonoids compounds are divided into several sub-classes, known as flavones, isoflavones, flavanones, flavonols, flavanols (catechins), and anthocyanidin. The basic structure for the flavonoids sub-class is given in **Figure 2-4**, in which the differences between sub-classes are associated with the substitutions in their benzene rings. In general, individual flavonoid compounds can be differentiated according to their end names, which indicate their sub-classes. For example, names ending in 'inidin' are often used to refer to compounds from the anthocyanidin group and names ending in 'etin' generally denote a flavonol (Cushnie and Lamb, 2005).

A large number of reports on flavonoid compounds and their antimicrobial activity has been published. The antimicrobial activity of many flavonoid compounds has been suggested to be due to their ability to form complexes with cellular proteins and bacterial membranes (Cowan, 1999; Guil-Guerrero et al., 2016). In general, highly lipophilic flavonoids such as catechin gallate are believed to exert their antimicrobial activity by binding with cell membranes, which leads to their leakage to intercellular components and cells lysis (Tsuchiya, 1999). Because of this unique property, some flavonoid compounds have been used in combination with commercial antibiotics to reduce bacterial virulence, as reviewed by Shin et al. (2018). Although currently there are limited studies available on the antiviral activity of flavonoids, earlier studies on flavonoid compounds, including quercetin, naringin, hesperetin and catechin, have shown that these flavonoid compounds are able to inhibit the infectivity and/or replication of the herpes simplex virus type 1 (HSV-I) and the respiratory syncytial virus (RSV) (Kaul et al., 1985).

With regard to the antimicrobial activity of flavonoid compounds, most attention has been given to compounds from the flavanol group. Flavanols in general can exist in two main forms: monomers (often referred to as catechins) and polymers (proanthocyanidins). Catechins can be found in high quantities in green teas; it is estimated that about 30% of dried tea leaf weight is catechins. Catechin compounds can be divided into two classes: non-galolyl catechins,

which include catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC); and gallolyl catechins, which include epicatechin gallate (ECg), epigallocatechin gallate (EGCg) and gallocatechin gallate (GCg). Gallolyl catechins (represented by the small 'g' in the catechin abbreviation) in general are formed through the esterification of catechins with gallic acids. The presence of gallate moiety in catechins' molecular structure has been shown to enhance catechins' lipophilicity, and the affinity of catechins to cell membranes. In general, gallolyl catechins are known to have higher antimicrobial activity than their non-gallolyl compounds. For instance, gallate catechins (i.e GCg, EGCg, ECg and Cg) have been shown to inhibit the foodborne pathogen *B. subtilis* more effectively at nanomolar concentrations compared to commercial antibiotics tetracycline and vancomycin, though no activity was observed in its non-gallolyl compounds (i.e. C and EC) (Friedman et al., 2006). A substantial body of information on catechins' antimicrobial properties has been reported, although currently their use is limited to EGCg (Taylor et al., 2009; Daglia, 2012). EGCg has been proposed as a good microbial inhibitor, especially against Gram-positive bacteria (Yoda et al., 2004; Taylor et al., 2009). In spite of their positive outcomes in microbial inhibitions, there are limited studies on catechins' antivirus properties. Nevertheless, the addition of EGCg to the influenza virus has been reported to reduce the binding of the influenza virus species to the viral host, which subsequently prevents viral infections (Nakayama et al., 1993).

Proanthocyanidins, also known as condensed tannins, are dimers, oligomers and polymers of catechins that are bound together by links between C4 and C8 (or C6) (Daglia, 2012). Wines and berries are recognised as the main source of proanthocyanidin compounds in the human diet. The addition of 1mg/ml berry extract into a bacterial growth medium has been shown to cause a significant reduction in three *E. coli* species within 4 hours of incubation (Puupponen-Pimiä et al., 2001). Using virus surrogates (FCV, MNV, MS2 and ϕ-X174), Su et al. (2010) demonstrated that the addition of 0.15 mg/ml of pure proanthocyanidins can cause up to 1-log reduction in the viral titer within 1 hour of incubation. Although the exact mechanism of proanthocyanidins' interaction with cells remains unclear, some researchers believe that proanthocyanidins exhibit their biological (i.e.: antimicrobial) activities by destabilising the

cytoplasmic membrane, increasing membrane permeability potential and also the leakage of intracellular components (Daglia, 2012).

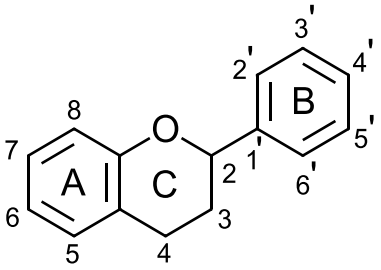



***Figure 2-3**: General structure of flavonoids*

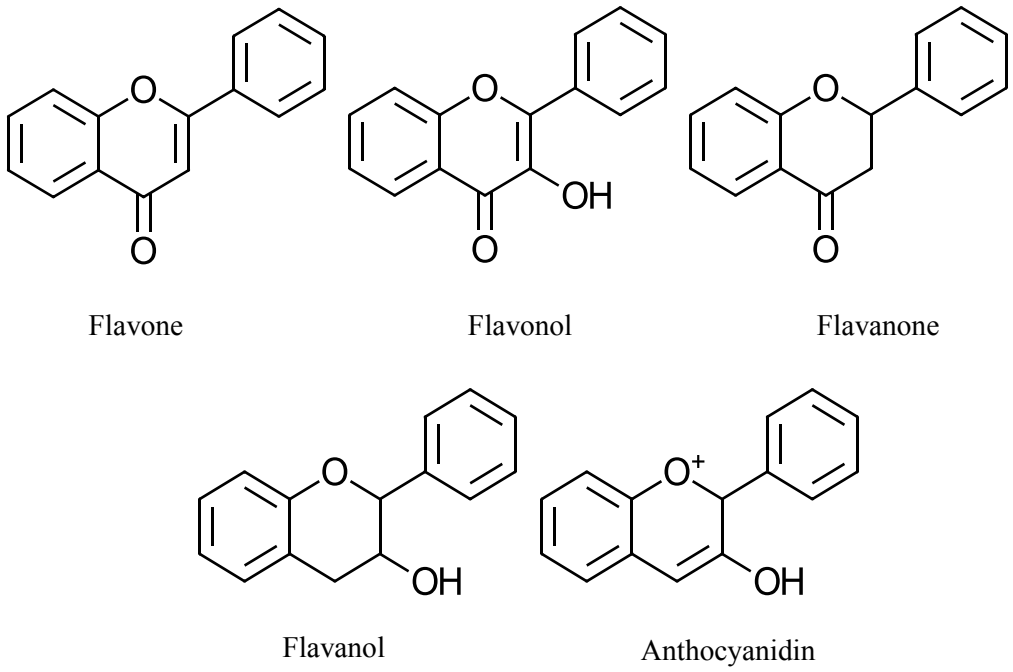
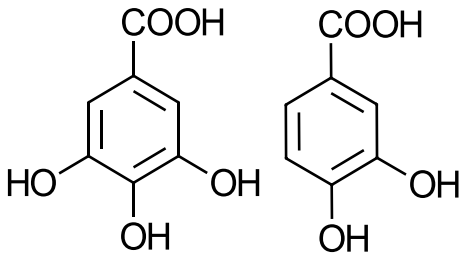



***Figure 2-4**: Basic structure of flavonoids groups*

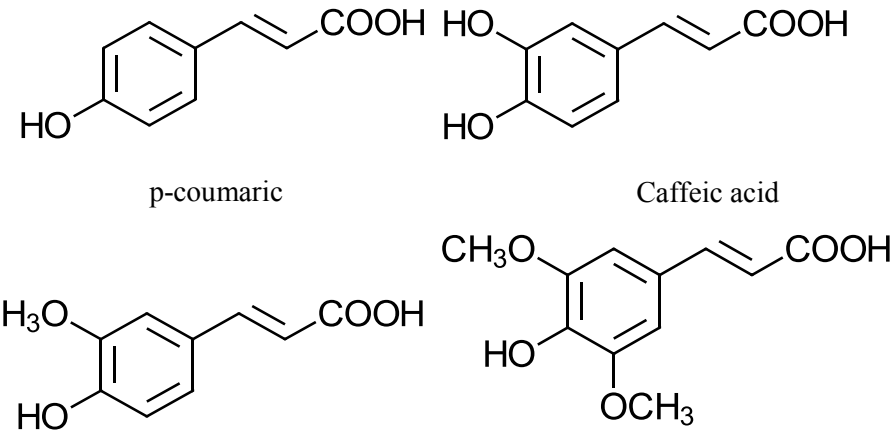
#### *2.2.1.2 Phenolic acids*

Phenolic acids can be divided into two different sub-classes (**Figure 2-5**) known as hydroxybenzoic acids and hydroxycinnamic acids. Caffeic, ferulic, p-coumaric and sinapic acids are examples of the most-studied hydroxycinnamic acids. This group of compounds can be found mainly in apricots, blueberries, carrots, cereals, pears, cherries, citrus fruits, oilseeds, peaches, plums, spinach, tomatoes and eggplants (Naczka and Shahidi, 2006). Meanwhile, hydroxybenzoic acid derivatives such as gallic acid and protocatechuic acid can be found in blueberries, cereals, cranberries and oilseeds (Naczka and Shahidi, 2006). Between these two sub-classes, compounds from hydroxycinnamic acids are thought to have higher antimicrobial activity than hydroxybenzoic acids due to their high hydrophobicity, which is associated with the presence of hydroxyl and methyl substitutions in their structure. Nonetheless, low-molecular-weight phenolic acids such as gallic acid are proposed to exert their antimicrobial effects through diffusion of the undissociated acid across the membrane, which leads to the acidification of the cytoplasm and in some cases, cell death (Campos et al., 2009; Guil-Guerrero et al., 2016; Wang et al., 2017). The antimicrobial activity of 13 phenolic acids against *Escherichia coli*, *Lactobacillus spp*., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* has been reported by Cueva et al. (2010). The strong antimicrobial activity of phenolic acids was noted at a concentration of 1000 µg/ml.




Gallic acid Protocatechuic acid

(a)




p-coumaric Caffeic acid

Ferulic acid Sinapic acid

(b)

***Figure 2-5****: Example of (a) hydroxybenzoic acids (b) hydroxycinnamic acids*

### 2.2.2 Structure-activity relationship of plant-derived compounds, and their effects on compounds' biological activity

It is generally understood that the biological activity of plant-derived compounds is highly dependent on their molecular structure (Cowan, 1999; Dorman and Deans, 2000). The presence of a hydroxyl group has been proposed by many researchers to be one of the major factors determining the antimicrobial activity of plant compounds (Cowan, 1999; Dorman and Deans, 2000). As such, the increased number of hydroxyl groups in phenolic compounds has been suggested to be the reason for their increased toxicity to microorganisms (Cowan, 1999). For instance, the one additional hydroxyl group in pyrogallol has been proposed to be the main reason for its higher inhibitory activity compared to the catechol group (Cowan, 1999). Although the exact mechanism remains obscure, compounds with a higher hydroxyl group are suggested to experience a higher degree of oxidation, forming hydrogen peroxide, which is responsible for the compounds' inhibitory activity (Kajiya et al., 2001)

The presence of a hydroxyl group has also been shown to be the essential parameter in the antimicrobial activity of carvacrol, a widely used plant-derived biocide. Using cymene and carvacrol as model compounds, Ultee et al. (2002) demonstrated that carvacrol had higher inhibitory activity against *B. cereus* compared to cymene. Carvacrol in general had an additional hydroxyl group in its structure, while cymene had a similar structure but lacked –OH in its structure. It is believed that the presence of a hydroxyl group in carvacrol allows it to act as an ion exchanger between the hydroxyl in carvacrol and the potassium in cell membranes, which leads to the leakage of intracellular components and lysis in cells (Ultee et al., 2002).

The importance of hydroxyl groups is believed to have lesser effects on compounds in the phenolic acids group. The effects of the hydroxylation number on phenolic acids' antimicrobial activity was studied by Sanchez-maldonado (2014), who found that the antimicrobial activity of hydroxybenzoic acids decreased significantly with an increasing number of hydroxyl groups. The results of their study showed that gallic acid, a compound with three hydroxyl groups, displayed weaker activity compared to syringic acid, a compound with one hydroxyl and two methoxy groups in its structure. Their findings therefore suggest that alkylation

on polyphenol benzene rings may have a more profound effects on plants' antimicrobial activity than hydroxylation. This is consistent with an earlier study by Cueva et al. (2010), which demonstrated that higher alkylation in fatty acids improved the antimicrobial activity of the compounds against bacterial cells. It is well known that a compound's alkylation improves its lipophilicity (Dorman and Deans, 2000). It has been proposed that the introduction of alkylation alters the distribution between the aqueous and non-aqueous phases by reducing the surface tensions between the compounds and the lipid structure of cell membranes. Therefore, the increase in compound lipophilicity has been thought to enhance compounds' partitioning ability in the cell membranes (Dorman and Deans, 2000). Indeed, higher antimicrobial activity towards *E. coli* and *S. aureus* by ferulic acid compared to gallic acid has been reported by Borges et al. (2013), who hypothesise that the antimicrobial activity of phenolic acids is dependent on the length of the hydrophobic chain.

A similar phenomenon was also observed in catechin compounds, i.e. EGCg and ECg, in which the substitution of gallate moiety to the parent catechin compounds has been shown to significantly increase the lipophilicity of the compounds (Nakayama et al., 2012). The role of a compounds' lipophilicity in antimicrobial activity has been studied previously by Caturla et al. (2003) using (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG). Using a model of phospholipid membranes, the effects of catechin compound partitioning on cell membranes was demonstrated, and the results of their study suggest that the presence of gallate moiety in catechins allows the compounds to localise (accumulate) in phospholipid membranes, due to their lipophilicity character. This disturbs the enzyme activities and protein functions in membranes, which eventually affects membrane function regulation.

Compound stereochemistry has also been reported to interfere with its activity, although conflicting results have been reported. In a study by Kajiya et al. (2001), a higher affinity of cis-catechins with cell membranes was reported, compared to trans-catechins. Using ECg (cis-catechins) and Cg (trans-catechins) as examples, the authors proposed that cis-catechins are able to penetrate more effectively into the cells' membrane due to the fact that the hydrophobic

domains of the structure are facing outwards, thus better contact between the compound and the cells' lipid structure can be achieved. Meanwhile, in the trans-catechins, the hydrophobic domain is located in the centre of the structure, and it is concealed by the hydrophilic domains of the A-ring, B-ring and galloyl moiety; thus it may interrupt the contact between the compounds and the lipid bilayer of the cells' membranes. However, in another study, higher antimicrobial activity by trans isomers, geraniol and nerol compared to cis isomers, α-pinene was reported by Dorman and Deans (2000).

## 2.3 Natural antimicrobials (biocides) from plants

### 2.3.1 Essential oils

Essentials oils in general are aromatic volatile liquids obtained from plant material (flowers, buds, seeds, leaves, twig bark, herbs, wood, fruit and roots)(Negi, 2012). Since most EOs consist of non-polar and volatile aromatic compounds (terpenes, alcohols, acetones, phenols, acids, aldehydes and esters), they are usually produced through expression, fermentation, effleurage or extraction, although the method of steam distillation is most commonly used for the commercial production of EOs (Burt, 2004). Generally, around 3000 EOs have been identified, of which 300 are used commercially. Some commercial EOs are recognised as 'GRAS' (generally recognised as safe) substances, and yet their use in many applications such as water disinfection is limited due to their hydrophobic character and their distinctive smell and flavour. Nevertheless, the most-studied EO components, carvacrol, is recognised as an important biocide agent being used in many hygiene applications, including hand washes and oral mouthwashes (Nostro and Papalia, 2012). It is proposed that the inhibitory mechanism of carvacrol is due to its interaction with cells' cytoplasmic membranes, which leads to an increase in membrane permeability (Nostro and Papalia, 2012).

In general, the inhibitory activity (i.e. antimicrobial and antiviral) of most EO compounds is dependent on pH, chemical structure and the concentration of EOs or the active compound, as well as the type of microorganism (Negi, 2012). The susceptibility of bacteria to EOs was shown

to increase with a reduction in pH; the lipophilic character of EO compounds was more significant at low pHs, which enables them to diffuse effectively through the lipid structure of bacteria cells (Burt, 2004; Balakrishnan et al., 2014). Apart from that, it also reported that the EO activity is dose dependent, with increased concentrations resulting in an increase in inhibitory activity (Ultee et al., 2002).

### 2.3.2 Plant extracts

Compared to EOs, plant extracts in general consist of higher proportions of polar polyphenols compounds, which can be obtained through simple extraction with organic and aqueous solvents. The antimicrobial activity of plant extracts has been reported to be due to the combined effects of different constituents in the extracts (Ikigai et al., 1993; Vuuren et al., 2011; Negi, 2012; Hübsch et al., 2014) and the generation of hydrogen peroxide (Kajiya et al., 2001; Arakawa et al., 2004). Although various in-vitro studies have been carried out to study the antimicrobial activity of plant extracts, and some promising results have been reported, very few of the extracts have been tested in real applications such as disinfection applications. Nevertheless, the application of different plant extracts has been widely tested in food applications, as reviewed by Negi (2012). However, it should be noted that extracts used in food application are exposed for long periods (24 hour incubation with the microbial species) therefore this cannot be used to simulate the practical scenario of plant extracts as a biocidal agent.

In a study by Negi (2012), the author also concluded that plant extracts in general had limited activity compared to their individual compounds. The reduced inhibitory activity of plant extracts may be due to the use of crude extracts in most of the studies. Crude extracts in general consist of compounds in glycosidic form (Mandalari et al., 2007), and as such the sugar moiety is known to promote the growth of certain bacteria species (Negi, 2012).

Owing to their polar character, plant extracts have also been shown to inhibit the growth of Gram-positive bacteria more effectively than Gram-negative bacteria. Thuille et al. (2003) demonstrated that extracts of grape kernel and evodia fruit exhibited higher activity towards Gram-positive cocci (*S. aureus* and *S. pyogenes*), the growth of which was inhibited after 30 - 90

min of incubation in grape kernel extract (0.5 - 1.5 mg/ml), and after 8 h in evodia extract (0.5 - 1 mg/ml), respectively.

### 2.3.3 Previous studies of plant-derived antimicrobials for disinfection applications

Numerous studies on plant antimicrobials have been documented, many of which describe plant-derived antimicrobials as active inhibitors of a broad spectrum of microbial species (Lewis and Ausubel, 2006; Taguri et al., 2006; Abreu et al., 2012; Taylor, 2013). However, there is only a small amount of literature currently available on their application as disinfectants of water or for other hygienic applications; many of them primarily focus on the use of EOs. In a study by Winward et al. (2008), the antimicrobial activity of 8 mixtures of EOs (origanum, thyme, tea tree, lemongrass, rosemary, clove bud, coriander and peppermint oils) and also eight major EO components (carvacrol, γ-terpinene, thymol, geraniol, eugenol, citronellal, linalool and limonene) were studied with regard to disinfecting total coliform from grey water. The results of their study showed that complete inactivation of total coliform was achieved after 60 minutes of treatment using origanum oils at 280 ppm. The results also revealed that no re-growth of coliform was observed in the treated grey water up to 14 days using origanum oils at 468 ppm. In another study using thyme oils, higher inactivation of *E. coli* was achieved compared to commercially used disinfectants (chlorine dioxide, ozonation) (Singh et al., 2003).

Using three different types of plant extracts – Moringa oleifera, Jatropha curcas and Guar gum, Pritchard et al. (2009) demonstrated the ability of plant extracts to purify shallow water in Malawi. The results of their study showed that almost 90% turbidity reduction was achieved using all three extracts, on well water with an initial turbidity of 49 NTU. Their study also demonstrated that almost 80% of coliforms were reduced using the extracts, although increases in pH were evidenced with increased extract doses. Similarly, the inhibitory effects of Neem oil extracts against total coliform in surface water in England and Nepal were studied by Matthews et al. (2009). The authors found that over 60% of total coliform was reduced after 60 minutes of treatment, though highly concentrated extracts (~17.1 g/L) were used in their study. In another

study, approximately 50% reduction in total coliform was achieved using 40 g/L of Luffa cylindrica fruit extracts after 60 minutes of disinfection treatment (Shaheed et al., 2009). It should be noted that such high concentrations as reported in Matthews et al. (2009) and Shaheed et al. (2009) are not practical for water application due to the undesired taste and colour associated with high concentrations of the extracts. Therefore, in some cases, using individual plant-derived compounds may be preferred to using raw extracts. For example, the inhibitory ability of individual plant-derived compounds, allyl isothiocyanate (AITC) to reduce heterotrophic plate count bacteria (HPCs) in Thames River water was reported by Mushantaf et al. (2012). Around 2-log reduction in HPCs was noted after 2 hours of contact time. In the same study, a higher reduction of *E. coli* species (~3-log reduction) was achieved using the same dose of AITC, tested for the same contact time.

## 2.4 Combining plant antimicrobials to improve their efficacy

In most plant antimicrobial studies, a great deal of attention has been directed to pure isolated compounds. It is well established that in order for plant-derived compounds to be considered as active antimicrobial agents, the MIC value of the compounds must be below 1000 μg/ml (Gibbons, 2004; Abreu et al., 2012). Individual plant compounds typically have MICs higher than 1000 μg/ml when tested against different bacterial species, particularly Gram-negative bacteria, and therefore most isolated plant-derived compounds continue to be considered as only weak inhibitors of bacterial growth. The failure of many antimicrobial studies to derive potent biocide agents from plant-derived compounds suggests that plants may have different mechanisms for preventing microbial diseases. The success of plants in resisting diseases has been speculated to be due to a complex mixture of plant secondary metabolites, which may perhaps act differently against various environmental stresses (Lewis and Ausubel, 2006; Taylor, 2013). Although attempts have been made in this regard, currently there is limited research on the combined effects of polyphenol compounds derived from the same complex natural material. One study on a combination of EGCg and EC, two major components from green tea extracts, proved that plant-derived compounds may indeed work in synergy (Kajiya et al., 2002). The

combined treatment of eugenol, cinnamaldehyde and thymol, has been shown to exhibit high inhibitory activity towards *E. coli*, although when used alone, eugenol has only weak activity (Pei et al., 2009).

Combinations between plant-derived compounds may lead to additive, synergistic or antagonistic effects. Synergy between compounds in general is based on the principle that compounds with different target sites, when combined together, could exhibit higher activity (Lambert et al., 2003). Using commercial cellulose wipes from Kimberly-Clark®, Catel-Ferreira et al. (2015) demonstrated that the addition of catechin significantly reduced the number of T4D viral titer, with up to 5-log reduction being observed after 1 h, compared to the performance of the wipes alone – this suggest that, by exploiting synergy interaction between natural compounds, a higher degree of protection against pathogenic microorganisms could be achieved.

In most cases, combination strategies are considered with the aim of: (1) reducing the dose required; (2) reducing the toxicity; and (3) preventing the emergence of resistance in bacterial species. Catechins are known to have higher MIC compared to commercial antibiotics (Taylor, 2013). Nevertheless, due to its ability to interact with cell membranes, addition of moderate concentrations (3–12.5 mg/L) of ECg has been shown to completely revoke β-lactam resistance in MRSA strains, inducing a reversible increase in susceptibility of MRSA bacteria to oxacillin from 512 mg/L to 1 mg/L (Stapleton et al., 2006). This study therefore highlighted that although some plant-derived compounds on their own are ineffective as antimicrobial agents, they are indeed a potent inhibitor when used in combination.

To summarise, while many plant-derived compounds may not have low-enough MIC values to be practical as water disinfectants and other hygienic applications due to the colour or taste that they would create in the water, the possibility of synergistic effects between compounds may allow lower concentrations of each of the individual compounds to be used, which could circumvent these taste and odour limitations.

## 2.5 Generation of hydrogen peroxide, and their relation to polyphenol biological activity

Reactions arising from hydrogen peroxide generation have been proposed as one of the mechanisms involved in the biological activity of plants. The generation of hydrogen peroxide has been postulated due to auto oxidation/degradation of plant compounds, following the equation showed in **Figure 2-6**. Various studies involving polyphenols have shown that biological activity (i.e. antimicrobial and antifungal activity) of plants and hydrogen peroxide production are correlated.

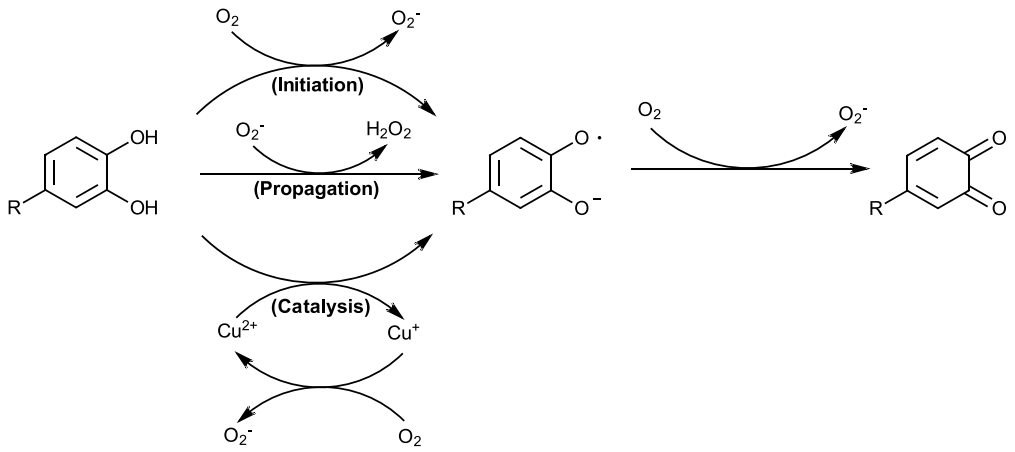



***Figure 2-6**: Proposed mechanism of hydrogen peroxide generation through polyphenols auto-oxidation.* Adapted from Akagawa et al.(2003)

Using Spearman's correlation rank, Chen et al. (2012) demonstrated that the antimicrobial activity of unprocessed honey was positively correlated to the formation of hydrogen peroxide, with higher *C. albicans* activity observed in honey samples containing a higher concentration of hydrogen peroxide. The study also indicated that the biological activity of the samples was significantly reduced when catalase was added to neutralise the hydrogen peroxide. Cui et al. (2012) also demonstrated that the addition of EGCg to *E. coli* cells produces similar morphology changes as when hydrogen peroxide is added to cells. Addition of increasing dose of catalase into solution containing EGCg has also been shown capable of reducing the bactericidal activity of EGCg against *E. coli* (Arakawa et al., 2004). In same study, the authors

also demonstrated that EGCg exhibits similar inhibitory activity to seven Gram negative and two Gram positive bacteria, including *E.coli* and *S. aureus* when compared to hydrogen peroxide at the same concentrations, suggesting that endogeneous hydrogen peroxide from plants may work in similar ways against bacterial cells.

A comprehensive study of factors that affect the generation of hydrogen peroxide by polyphenols has been reported by Akagawa et al. (2003). In the study, the authors concluded that several factors such as compound structure, pH and incubation time were shown to influence the formation of hydrogen peroxide. The main findings from the study are summarised in **Table 2-2**.

***Table 2-2:*** *Factors affecting hydrogen peroxide generation*

| Factors | Findings |
|---|---|
| **Compounds structure** | OH-substitution in phenols have been shown to influence the production of hydrogen peroxide by polyphenols. The study by Akagawa et al. (2003) indicates that the hydrogen peroxide generating property of polyphenols is related to the auto-oxidation at ortho- and para- positions. For instance, the addition of –OH in the compounds structure increased the formation of hydrogen peroxide following the order of pyrogallol >1, 2, 4-benzenetriol > hydroquinone > pyrocatechol.<br>Moreover, the addition of gallate moiety in the catechins also increases the formation of hydrogen peroxide. The order of formation of hydrogen peroxide in catechins is given as follows: EGCg > ECg ≈ EGC > EC > C. |
| **pH** | Polyphenols in general autooxidise at higher rates at higher pH values. Nevertheless, in the study by Akagawa et al. (2003) higher formation of hydrogen peroxide was detected when the pH of the solution was in the range of 7-9. At pH above 9, no significant change in hydrogen peroxide formation was noted. |
| **Media** | Using deionised and phosphate buffer as medium, Akagawa et al. (2003) found higher generation of hydrogen peroxide in phosphate buffer, with the increase in hydrogen peroxide directly proportional to the incubation time. The results suggested that accelerated formation of hydrogen peroxide in phosphate buffer medium was likely due to the presence of trace metal ions such as iron, which influence the degradation rate of compounds. |

## 2.6 Availability of phenolic compounds from plant by-products/ waste materials

Increased awareness from the public of the nutritional value of fruits has indirectly led to an increased demand and consumption of agricultural products. Processing agricultural products often involves the separation of its valuable parts (the main products) from the unwanted parts such as seed, kernel, shells and peels, which produces a significant amount of waste. For example, in the citrus industries, where about $15\times10^6$ tons of waste are generated during the processing of the fruit, the residues constituted 50% of the original whole fruit mass (Ayala-Zavala et al., 2011). Several methods have been proposed by researchers to utilise the generated by-products, including the conversion of this waste material into antimicrobial agents (Guil-Guerrero et al., 2016).

Studies of plant by-products have shown that waste material from plants in general has a higher amount of bioactive compounds such as polyphenol, compared to their edible part. For instance, peels from apples, peaches and pears have been reported to contain twice the phenolic contents of their fruit part (Gorinstein et al., 2001). Similarly, Someya et al. (2002) also showed that banana peel contains about 158 mg/100g DW of gallocatechin compared to 29.6 mg/100 g DW in the pulp. The abundance of bioactive compounds in the dermal parts of plants has been suggested to be part of the defence mechanism of plants against pathogenic diseases (Sanchez-maldonado, 2014). Studies have shown that extracts and phenolic compounds recovered from plant by-products have huge potential as antimicrobial agents. For instance, mango kernel extract was reported to have higher antimicrobial activity than crude green tea extracts. It inhibited two pathogenic bacteria, *Campylobacter jejuni* and *Yersinia enterocolicia*, at an MIC of 100 and 50 µg/ml respectively (Kabuki et al., 2000). Similarly, Mandalari et al. (2007) have also shown that individual flavonoids isolated from bergamot peels exhibit higher inhibitory activity against Gram-negative bacteria such as *E. coli*, *Pseudomonas putida* and *S. enterica*, with MIC values ranging from 200-800 µg/ml. An *in vivo* study of orange peels also showed that the addition of the extracts at a concentration of 0.5% completely inhibited *E. coli* growth at an initial concentration of $10^7$ cfu/ml (González-Gómez et al., 2014).

Therefore, plant by-products may provide cheap sources of antimicrobial agents that could be used as potential inhibitors against various pathogenic organisms. The utilisation of these bioactive compounds from agricultural by-products as antimicrobials could help to solve some problems encountered by agricultural-intensive countries in terms of reducing the cost of their waste management and deriving a value from the waste. In addition, this effort also could help the biocide industries to achieve their goals in producing low-cost biocides that are safe in nature.

## 2.7 Bananas – an introduction

The banana, scientifically called *Musa spp*, is a tropical fruit genus naturally found in tropical countries such as Brazil and India. The human-driven production of bananas is dominated by India, China, Uganda, Ecuador, Philippines and Nigeria (Padam et al., 2012). Although this tropical fruit is cultivated mainly in hot-climate countries, the European Union and United States are the largest importers of bananas, accounting for 29% and 27% of the global banana market, respectively. The total production of bananas was estimated to be around 117.9 million tonnes in 2015, an increase of 3.7% compared to 68.2 million tonnes in 2000 (FAO, 2018). In general, approximately 1000 varieties of bananas are cultivated across the world, although the most commercialised banana species are from the Cavendish species, which accounts for roughly 47% of global banana production (FAO, 2018).

Bananas are normally cultivated for their fruits, hence banana farming contributes significant amounts of waste products, such as bagasse, peel, seeds and unwanted processing parts (Rebello et al., 2014). Banana peel, which constitute 30% of the total fruit weight, is one of the major waste products from banana industries. Some effort has been made to convert this waste material into value-added products, including using them as bio colourants (Padam et al., 2012), raw material for animal feeds (Aurore et al., 2009; Padam et al., 2012) and also for the adsorption of heavy metals (Annadural et al., 2003). Despite all this effort, banana peels are still an underutilised waste material. Banana peels are known to be a rich source of phenolic compounds, with some reported antimicrobial activities on various microbial species. In fact, due to their

antiseptic properties, banana peels have been shown to be an effective wound treatment, used for reducing swelling and infections (Pereira and Maraschin, 2015).

### 2.7.1 Bioactive compounds in banana peels

Different studies on banana peel extracts have been carried out, and they have reported the presence of polyphenol mixtures such as catechins, phenolic acids, anthocyanin, dopamine and fatty acids. It has been suggested that banana peels in general have higher levels of phenolic compounds compared to the edible part of the fruit. Someya et al. (2002) evaluated the gallocatechin content in the peel and pulp of banana, and their findings showed that approximately 158 mg/100 g dry wt. can be found in banana peel compared to 29.6 mg/100 g dry wt in the fruit pulp. In the same study, the authors also identified the presence of catechin and epicatechin, two of the compounds that can be found in green tea extracts, which have been associated with the antimicrobial ability of green teas extracts. A recent study by Septembre-Malaterre et al. (2016) reported the presence of epigallocatechin gallate (EGCg) in Dwarf Cavendish banana species. This was quantified using ultra-high-performance liquid chromatography analysis coupled with diode array detection and electrospray ionisation-mass spectrometry.

Rebello et al. (2014) have reported the presence of (+)-catechin, (−)-epicatechin and (−)-gallocatechin and three B-type procyanidin dimers – procyanidins B1, B2 and B4 – in banana peel extracts obtained through maceration with a mixture of methanol, water and formic acid. The study also reported that banana peel extracts in general had approximately 29 mg of gallic acid per gram of their dry weight (Rebello et al., 2014). An earlier study on anthocyanins in banana by Simmonds (1954) concluded that the anthocyanin content in bananas varied depending on their genus. Recent study on anthocyanins in banana extracts has been carried out by González-Montelongo et al. (2010) who determined that an amount of 139 µg cyanidin 3-glucoside equivalents/100 g dry wt. was present in the acetone extracts of banana peels extracted at 25 ºC for 1 minute maceration period. The authors also indicated that the extraction of the

banana peels with methanol contributed significantly to the increased dopamine content in the extracts (González-Montelongo et al., 2010).

The presence of dopamine in banana peel extracts was reported in an earlier study by Kanazawa and Sakakibara (2000), which determined dopamine levels to be in the range of 80-560 mg per 100 g in peel, and about 2.5-10 mg per 100 g in banana pulp. Dopamine is a biogenic amine in the catecholamine class. Biogenic amines have been found to be one of the key groups in plant metabolic pathways, responsible for rendering pathogen resistance (Pereira and Maraschin, 2015). Because of that, it is generally accepted that the concentration of dopamine content in banana reduces tremendously during the ripening stage, evidenced by the formation of black pigments in the banana peel (Kanazawa and Sakakibara, 2000).

Mokbel and Hashinaga (2005) studied the variation in fatty acids extracted from green banana peels and reported that ß-sitosterol, malic acid and succinic acid isolated from banana extracts had positive inhibition towards *S. aureus, B. subtilis, B. cereus, S. enteritidis* and *E. coli* at concentrations varying from 140 to 750 mg/l. The mechanism of bacterial inhibition by fatty acids has been reported by Desbois and Smith (2010), who proposed that the longer chain fatty acids in general exert higher microbial inhibition than the shorter chain fatty acids.

The composition of bioactive compounds in the peels of plantains have been studied (Passo Tsamo et al. 2015). The results revealed that plantain peels in general consist of high amounts of flavonol compounds, whereas hydroxycinnamic acids are more predominant in the pulp. Similarly, the authors also identified the presence of epicatechin in their plantain samples.

### 2.7.2 Antimicrobial studies on banana peel extracts

Many studies on banana peel extracts have demonstrated that the susceptibility of bacterial species to banana peels is generally dependent on the solvents used during the extract's preparation. For example, in a study by Rattanavichai and Cheng (2014), the antimicrobial ability of aqueous extracts of banana peels was used to inhibit seawater pathogens such as *Vibrio parahaemolyticus, V. alginolyticus, Photobacteria damsala* and *L. garvieae.* The results of the study revealed that the addition of banana extracts inhibited seawater pathogens, with higher

inhibitions being observed on Gram-negative pathogens than on Gram-positive ones. In another study by Matook and Fumio (2005), however, no antimicrobial activity was observed on Gram-positive (*S. aureus*, *B. subtilis*, *B. cereus*) and Gram-negative bacteria (*Salmonella enterica*, *E. coli*), when water extracts of green and yellow banana peels were used. However, when tested using the ethyl acetate fraction of banana extracts, higher inhibitions towards Gram-positive bacteria were observed compared to Gram-negative bacteria (Mokbel and Hashinaga, 2005). A recent study by Siddique et al. (2018) also showed that significant activity on *S. aureus*, *B. subtilis*, *P. aeruginsa* and *E. coli* was noted using ethanolic extracts of banana peels. Based on the above examples, it can be concluded that the antimicrobial activity of banana extracts can be influenced by the type of solvents used during the extracts' preparations, with organic solvents seeming to be the better choice for obtaining extracts with a higher antimicrobial activity.

## 2.8 Assessment of antimicrobial activity in plant compounds

In general, no specific protocol is available to assess the antimicrobial activities in plant extracts or in their individual compounds. Nevertheless, two general antimicrobial susceptibility methods are often used and cited when testing the *in-vitro* study of plants' antimicrobial activity: the disk diffusion and broth or agar dilution methods.

### 2.8.1 The disk diffusion method

The disk diffusion method is the earliest method developed for testing the susceptibility of antimicrobial agents. Due to its simplicity, it is often used in many microbiology labs for antimicrobial study routine checks. The disk diffusion method in general is based on the principle of the diffusion of antimicrobial agents into a medium inoculated with specified amounts of bacterial species. The observation of antimicrobial activity is carried out after the optimum incubation of the bacterial species has been achieved. Test samples are considered to possess antimicrobial ability when there is a clear inhibition zone surrounding the disk. Because of its simplicity, the disk diffusion method is often used for screening newly found antimicrobial agents. Nonetheless, this method has a limitation as it cannot be used to determine the inhibitory

concentration of antimicrobial agents, because it is almost impossible to know the amount of the agent that has diffused through the agar. In addition, this method is also unsuitable for compounds with low diffusivity as they might give false results regarding the inhibition diameter. Despite its weaknesses, the disk diffusion method is still considered to be an important method for determining antimicrobial activity in antimicrobial agents such as antibiotics and other naturally occurring substances used to kill and inhibit bacterial growth (Gordon and Wareham, 2010; Betts et al., 2011).

### 2.8.2 Dilution methods

Dilution methods are used to determine the MIC values of antimicrobial agents (Yoda et al., 2004; Sarker et al., 2007; Gordon and Wareham, 2010; Monte et al., 2014). In contrast to the disk diffusion method, the dilution methods provide a quantitative estimation of the concentration required to inhibit growth. The resulting MIC values are defined as the lowest concentration assayed that completely inhibits the growth of the microorganism tested. The concentration of the test samples is often expressed in µg/ml or mg/L. Depending on the medium used for the bacterial growth, this method can be divided into two: broth and agar dilution.

#### *2.8.2.1 Broth dilution method*

Broth dilution methods can be divided into two: macrodilution, which requires at least 2ml of test volume, and microdilution, which is normally carried out using 96 well plates, with a maximum volume of 200 µl (Monte et al., 2014) . Between these two methods, the latter is more popular among researchers because only small amounts of antimicrobial agents are required (Balouiri et al., 2016), and it can be carried out simultaneously on different kinds of antimicrobial agents. The basic principle of the dilution method is based on the preparation of a two-fold dilution of antimicrobial agents in a broth medium, followed by the addition of the agents to a known population of bacterial cells (normally in $10^5$ colony-forming units (cfu/ml). Antimicrobial activity is evaluated by determining the concentration that completely inhibits the

microbial growth, after incubation. The MIC endpoint is usually read using a microplate reader, or by using a UV spectrophotometer for the macrodilution method. However, recently, the application of dyes has received much attentions among researchers for the purpose of aiding the MIC reading. Several dyes such as tetrazolium salts, 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2,3-bis {2-methoxy-4-nitro-5-[(sulfenylamino) carbonyl]-2H- tetrazolium-hydroxide} (XTT) and also sodium resazurin dyes (Kumarasamy et al., 2007; Sarker and Nahar, 2009) are often used for MIC visualisation.

Although this method seems simple and straightforward, some factors are known to interfere with the MIC endpoints. According to Wiegand et al. (2008) these are:

a) Medium

The susceptibility of some bacteria to certain antimicrobial agents, particularly antibiotics, is known to be influenced by the number of $Ca^{2+}$ and $Mg^{2+}$ ions in the broth medium. A commonly used broth medium, tryptone soya agar (TSA), in general contains insufficient amounts of divalent cations, which can give a false MIC value. The broth therefore needs to be supplemented with divalent cations during the MIC testing. The final concentration should be 20–25 mg $Ca^{2+}$ and 10–12.5 mg $Mg^{2+}$ per litre of broth. This reflects the amount of divalent cation concentration in blood. Nevertheless, the medium has been standardised by the Clinical and Laboratory Standards Institute (CLSI), and a cation-adjusted MHB is recommended as the standard medium used for MIC testing (CLSI, 2012).

b) Inoculum

It is generally accepted that the inoculum size is the critical parameter in determining MIC endpoints. Higher inocula can lead to an increase in the MIC value after the incubation. The recommended inoculum size for broth dilution testing is $5 \times 10^5$ cfu/ml. Moreover, since old bacteria cultures are known to form aggregate after days of preparation, and as this might reduce the susceptibility of bacteria to the antimicrobial agents, a fresh, pure culture is suggested for the preparation of the inoculum.

Due to their sensitivity, several control strains as shown in **Table 2-3** have been proposed by certified susceptibility agencies such as CLSI to check the accuracy of the MIC determination.

***Table 2-3**: Control strains used for antimicrobial susceptibility testing*

| **Bacteria species** | **ATCC control strains*** | **Identical strains to ATCC*** | **NCTC control strains**** |
|---|---|---|---|
| ***Escherichia coli*** | ATCC 25922 | NCTC 12241, CIP 76.24, DSM 1103 | NCTC 10418 |
| ***Pseudomonas aeruginosa*** | ATCC 27853 | NCTC 12924, CIP 76.110, DSM 1117 | NCTC 10662 |
| ***Staphylococcus aureus*** | ATCC 25923 | NCTC 12981, CIP 1033429, DSM 2569 | NCTC 6571 |
| ***Enterococcus faecalis*** | ATCC 29212 | NCTC 12697, CIP 103214, DSM 2570 | - |
| ***Haemophilus influenza*** | ATCC 49247 | NCTC 12699 | NCTC 11931 |

*adapted from Wiegand et al. (2008), **adapted from Andrews (2001)

#### *2.8.2.2 Agar dilution method*

Similar steps to those described in broth dilution are normally followed for agar dilution, with the exception that agar is used for microbial growth instead of a broth medium. This method is however less popular than broth dilution. Nevertheless, agar dilution is often recommended for the determination of MIC in fastidious organisms such as anaerobes and *Helicobacter* species (Balouiri et al., 2016).

# Chapter 3 MATERIALS AND METHODS

In this chapter, the materials and methods used to assess the antimicrobial activity of the raw material, banana peels, will be discussed. In order to allow better understanding of the experimental work conducted in this project, this chapter is divided into five sections. In the first section (3.1), the processing steps for producing the extract and compound identification using High Pressure Liquid Chromatography (HPLC) will be explained. The second section (3.2) will discuss the experimental steps involved in determining the antimicrobial activity of the extracts and the identified compounds, including the disinfection protocol used for the study. The third section (3.3) will explain the method used to assess the combination activity between the compounds, while 3.4 focuses mainly on the application of MS2 coliphage as a viral surrogate in testing the antiviral activity of the extracts and the individual compounds. And finally, in the last section (3.5) the methods used to quantify the hydrogen peroxide generated by the extracts/compounds during the disinfection study will be discussed; this is because one of the proposed modes of action for plants' biological activities is the generation of hydrogen peroxide.

## 3.1 Extraction and compound identification

### 3.1.1 Plant material

In this study, yellow to greenish Musa Cavendish bananas at index no 4 to 5 (**Figure 3-1**) were purchased from local supermarkets in South Kensington, London. The selection of the bananas was based on the fact that young plant material in general has a higher phenolic content, as observed through the absence of any black pigmentation in the skins (Sanchez-maldonado, 2014). In addition, as bananas are also normally consumed at these two maturation stages, this simulates the actual condition whereby most banana peels are being produced as a waste product. Geographic locations also are known to influence the availability of plant bioactive compounds (Arvanitoyannis and Mavromatis, 2009), only bananas of Ecuadoran origin were chosen as the plant material in this study, for consistency.

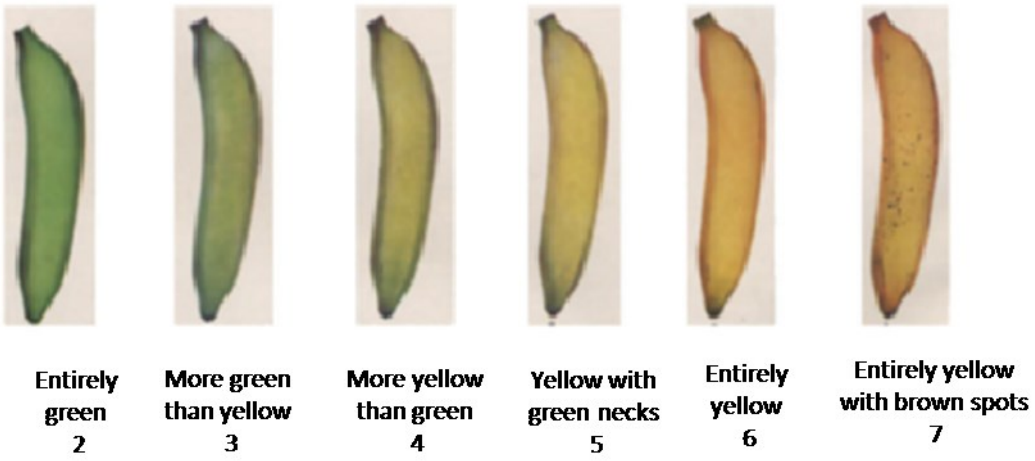



***Figure 3-1**: Banana peel colour index.* Adapted from Aurore et al. (2009)

### 3.1.2 Material processing

The peels were separated from their edible part (the pulp) using standard laboratory scissors. They were then cut into approximately 1cm x 1cm squares. The peels were washed using reverse osmosis (RO) water to remove any impurities or dirt from the samples. After washing, the peels were separated into two groups for drying purposes. Group 1 banana peels were dried using oven drying at 40$^{o}$C, while group 2 peels were evenly distributed in a freeze-dryer under reduced pressure. The peels were left to dry for at least 4 days until a constant weight was achieved (approximately 90% moisture reduction). The dried peels were pulverised using Wahl blender (James Martin, United Kingdom) for 1 minute and sifted through ≤355µm sieve. The powdered material was kept in amber glass bottle and stored at -20$^{o}$C fridge until used for analysis.

### 3.1.3 Initial screening for solute/solvent ratio

At the beginning of this project, preliminary testing on extraction conditions, related to the ratio of the solute/solvent, was conducted to determine the most economical combination in terms of the solvent used and the amount of extractable solids. However, the addition of 1g to 6g

of freeze-dried banana powder to 50 mL of methanol had no statistical significance on the yield recovery ($p>0.05$), suggesting that this factor did not interfere with the rate of solubilisation of phenolic compounds in the dried banana peels. Therefore, in the preparation of the crude extracts, 10g of peel powder was added to 200mL of the methanol as a ratio between 2:50 and 3:50 gives the highest yield recovery. Full results on this initial testing are provided in the Table A *1*.

### 3.1.4 Crude extract preparation

Although different methods have been proposed by researchers for producing plant extracts, the conventional method of extraction was chosen in this study due to the fact that most of the phenolic compounds could be easily solubilised in organic solvents. Although this conventional method is known to have the drawbacks of being a time- and solvent-consuming technique compared to advanced extraction methods such as supercritical fluid extraction (SFE), it continues to be popular among researchers as it can be used as the primary screening for bulk materials (Sarker and Nahar, 2009).

The optimisation of banana peel extraction has been reported previously by González-Montelongo et al. (2010) using varying polarities of solvents such as methanol, ethanol, acetone and water. Of these, they showed that methanol was the most effective solvent in extracting the bioactive compound from banana peel. Therefore, extraction using methanol as the main extractant was chosen as the method for obtaining the crude (raw extracts without fractionation) extracts from the banana peels in this study.

The procedure for the extraction was as follows: peel powder (10g), obtained through oven drying and freeze drying, was extracted in methanol (200mL) for 1 hour, 3 hours, 6 hours, 12 hours, 24 hours and 48 hours' extraction time. The extraction procedure was carried out at room temperature (±20 $^{o}$C) using Erlenmeyer flasks with stoppered caps to prevent the evaporation of the solvent. To ensure complete dissolution between the solute and the solvent, the content was stirred using a magnetic stirrer at 100 rpm. At the end of each extraction time, the supernatant was separated from the peel powder using Whatman No 1 filter paper.

Afterwards, the collected supernatant (extract) was transferred to a 1 litre evaporating flask (Buchi®) and concentrated under reduced pressure using a rotary evaporator (Buchi®). The water bath in the rotary evaporator was set at 40$^{o}$C to prevent further decomposition of heat-sensitive bioactive compounds (Sarker and Nahar, 2009). The weight of the dried extract was measured and the extract was re-dissolved in a solvent to a desired final concentration. The extract was transferred to amber glass bottles and kept at -20$^{o}$C fridge until required for further analysis. The illustrative flow-diagram of this extraction procedure is provided in **Figure 3-2.**

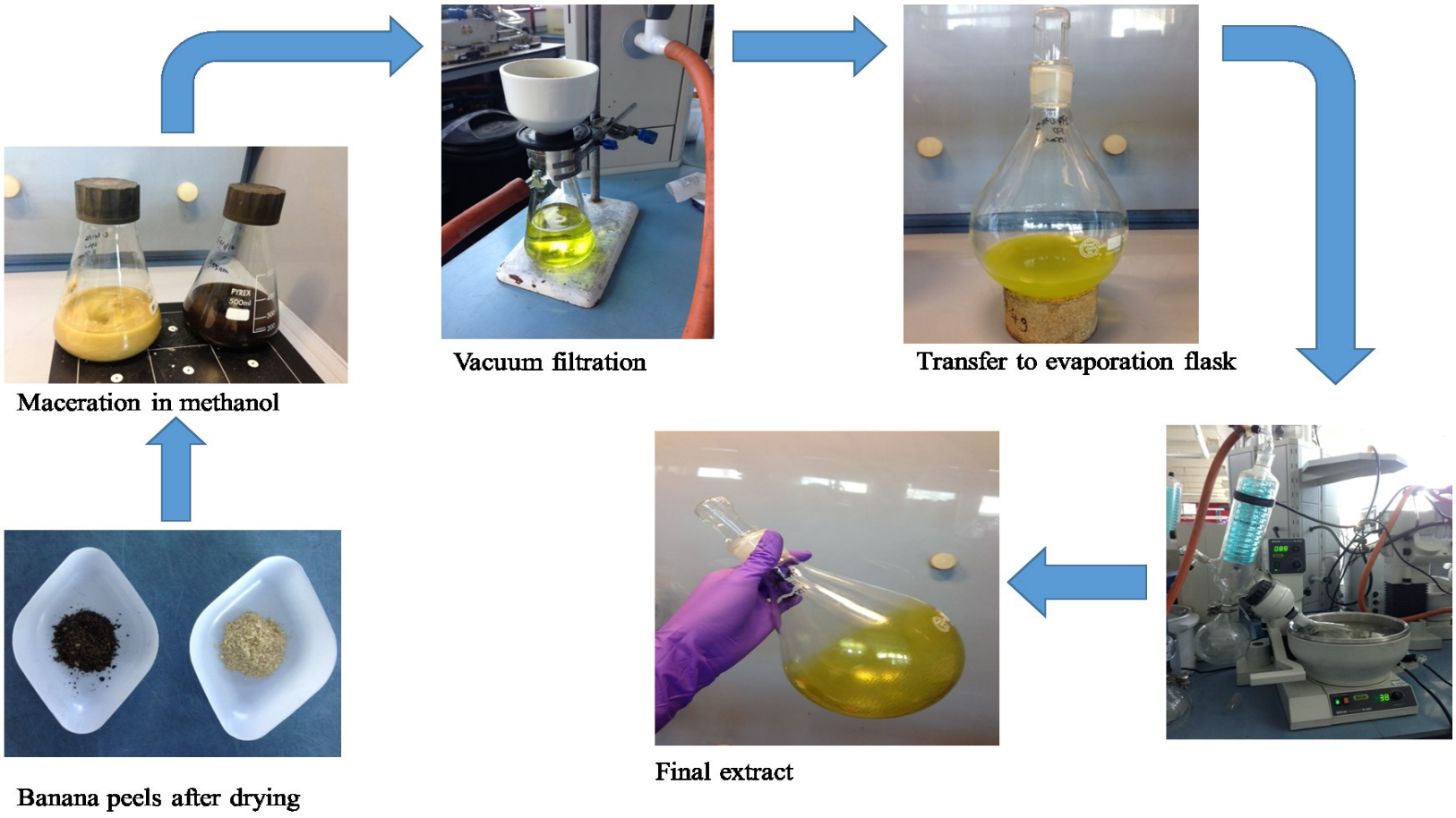



***Figure 3-2:*** *Schematic diagram for extraction procedure.*

The extract was re-dissolved in methanol for HPLC analysis. For microbiology analysis, the extract was prepared in dimethyl sulphoxide (DMSO).

### 3.1.5 Fractionation of the crude extracts

Crude extracts in general consist of a complex mixture of compounds with different polarities. The separation between the compounds can be achieved through fractionation. In this study, liquid-liquid extraction was chosen as the fractionation step as this method is simpler to carry out (Sarker and Nahar, 2009), and also it provides more consistent results between each batch than column chromatography.

Using a separating funnel (1000 ml), the crude methanolic extracts (100 mg/ml) were fractionated using equal amount (100 mL) of hexane and methanol to separate most of the lipophilic components. The hexane fraction was then concentrated by drying using a rotary evaporator and tested for biological activity and chromatography analysis. Following this step, the volume of aqueous extracts was adjusted to 100 ml and partitioned with an equal amount of chloroform (100 ml) to separate the remaining apolar compounds from the aqueous extracts. All fractions were collected and concentrated by drying using the rotary evaporator (Buchi®). The water bath in the rotary evaporator was set at 40$^{o}$C.

### 3.1.6 Compound identification using High Performance Liquid Chromatography (HPLC) analysis

#### *3.1.6.1 Chemicals and reagents*

The following standard compounds – gallic acid (GA), catechin (C), epicatechin (EC), epigallocatechin gallate (EGCg), gallocatechin (GC), and cyanidin 3-O glucoside – were purchased from Sigma Aldrich (United Kingdom). The details and catalogue number for each standard are shown in **Table 3-1** below. Methanol (HPLC grade) was supplied by Sigma Aldrich, while acetonitrile and water, also in HPLC grade, were purchased from VWR chemicals (United Kingdom). Acetic acid solution 1% was prepared manually by adding 1 ml acetic acid solution (cat no. 45754-100ML-F-D, Sigma Aldrich) to 99 ml HPLC-grade water.

***Table 3-1**: Details of the selected phytochemicals used in this study*

| Compound | Catalogue number | Supplier |
|---|---|---|
| **Gallic acid** | 91215 | Sigma Aldrich |
| **Catechin** | 43412 | Sigma Aldrich |
| **Epicatechin** | E4018 | Sigma Aldrich |
| **Epigallocatechin gallate** | E4268 | Sigma Aldrich |
| **Gallocatechin** | 01388 | Sigma Aldrich |
| **Cyanidin 3-O glucoside** | 44689 | Sigma Aldrich |

#### *3.1.6.2 Sample preparation*

Dried extracts were re-dissolved in methanol to a desired concentration, mostly in 100 mg/ml. For quantification of compounds, the extracts were further diluted using 1% acetic acid to a final concentration of 1000 µg/ml and filtered through a 0.45 µm polypropylene filter syringe (Whatman, Fisher Scientific) before being analysed using high pressure liquid chromatography (HPLC). The standard compounds were treated similarly, by dissolving the compounds in methanol to a stock concentration of 1000 µg/ml. Then, they were diluted to working stock using 1% of acetic acid before being analysed in the HPLC. In this study, 1% acetic acid was chosen as the diluent instead of methanol or water to minimise the HPLC back pressure and improve the miscibility between the sample and mobile phase during compound separation. The selection of this diluent was also made as it helped to solve the background noise problem at the beginning of this study and subsequently minimised the time taken for the HPLC system to stabilise.

#### *3.1.6.3 Compound separation*

Waters 2695 separation module was used for compound separation. This consisted of a binary pump, photodiode array detector and column temperature controller. Briefly, 10 µl of the sample was injected into a Luna C18 column (2.1 x 250mm, 5µm particle size) at a flow rate of 0.2 ml/min. Two solvent systems consisting of absolute acetonitrile (A) and 1% acetic acid (B)

were used in the separation process. The selection of acetonitrile as the main organic element during compound separation was made based on its ability to reduce the noise and band broadening compared to methanol (Řehová et al., 2004; Spáčil et al., 2008). The use of acetonitrile was also found to solve the noise problem in the HPLC system at the beginning of this project. During compound separation, the following gradient programme was used to allow complete separation between the compounds of interest: 0-10 min, 2-30 % A; 10-20 min, 30-50 % A; 20-25 min, 50-30 % A; 25-30 min, 30-20% A; 30-40 min, 20-2% A. The column temperature was maintained at 25$^{o}$C as recommended by Spáčil et al. (2008) for the separation of the phenolic compounds. The compound detection was observed at 280 nm wavelength, which allowed simultaneous detection of simple phenolics (He and Giusti, 2011). The total run for each sample was set for 40 minutes, and a delay time of 10 minutes was used prior to the next injection in order to equilibrate the separation system to its initial gradient, and also to flush out any remaining carryover from the samples. This HPLC condition was kept constant for all the samples and standards. Empower 2 software was used for chromatography quantification and data integration.

## 3.2 Antimicrobial study

### 3.2.1 Medium preparation

Three growth media were used this study: tryptone soya agar (TSA), tryptone soya broth (TSB) and Mueller Hinton Broth (MHB) cation adjusted. Otherwise stated, TSA was the medium used for bacteria enumeration. TSB was used during the preparation of overnight bacteria stocks. MHB was the broth medium used during the MIC culturing. The selection of the mediums was made based on the recommendation by CLSI (CLSI, 2012). The preparation for the mediums was as follows:

Tryptone soya agar was prepared by dissolving 40 grams of powder medium (Oxoid, Fisher Scientific) into 1 litre of RO water. The prepared medium was then heat sterilised in an autoclave (Priorclave) at 121°C for 20 minutes. After sterilisation, the medium was left to cool

down at room temperature to 40-50°C. The TSA agar plates for the experiment were prepared by pouring the sterilised agar into 55 mm culture plates (VWR, United Kingdom). All of the steps involving the agar preparation after autoclaving were carried out in the biosafety cabinet to avoid any bacterial contamination. The prepared agar plates were kept at 4°C in a fridge, and used within two weeks of production.

Tryptone soya broth was prepared by adding 30 grams of powder medium (Oxoid, Fisher Scientific) to 1 litre of RO water. The mixture was mixed and heat sterilised in an autoclave at 120°C (Priorclave) for 20 minutes. The sterile TSB medium was kept in the fridge at 4°C and used within a month of production.

Mueller Hinton Broth (MHB) cation adjusted is a medium used during MIC determination. The selection of the medium was based on standard protocol for antimicrobial susceptibility testing, as approved by the CLSI. The medium was prepared by adding 11 g of the medium (Oxoid, Fisher Scientific) to 500 mL of RO water. The medium was heat sterilised as described above. The sterile broth was kept at 4°C in a fridge and used within two weeks of production to prevent any contamination between testing.

### 3.2.2 Phosphate buffered saline (PBS) preparation

The phosphate buffer solution (PBS) used in this study was prepared using the following steps. It should be noted that the disinfection experiment in this study was conducted using PBS as the test medium, therefore a concentrated stock (10x) of PBS was prepared, and prior to any experiments involving its application, the 10x PBS stock was diluted to 1x concentration using RO as the diluent. The 10x concentrated PBS stock was prepared by adding 80 g sodium chloride (Sigma Aldrich, USA), 2 g potassium chloride (Sigma Aldrich, USA), 14.4g sodium phosphate dibasic (Sigma Aldrich, USA), and 2.4 g monopotassium phosphate (Sigma Aldrich, USA) into 800 mL RO water in a 1 litre volume Schott Duran reagent bottle. The mixture was thoroughly mixed until complete dissolution of the salts was achieved. Using a Fisherbrand Hydrus 500 pH meter, the pH of the solution was adjusted to 7.2-7.4 by adding 1M sodium hydroxide (NaOH)

or 1M hypochloric acid (HCl) to the solution. Following this, approximately 200 ml of RO water was added to the PBS solution to a final volume of 1 litre. The prepared 10x PBS stock was heat sterilised using an autoclave (Priorclave) at 120$^{o}$C for 20 minutes.

### 3.2.3 Tested bacteria

Two types of bacteria were used in this study. Gram-negative bacteria, *Escherichia coli* strain number NCTC12241 and Gram-positive bacteria, *Staphylococcus aureus* NCTC6571 were obtained from the National Collection of Type Cultures (NCTC), purchased from Public Health England. These two strains were selected based on the recommendation made by Public Health England (Andrews, 2001).

### 3.2.4 Preparation of bacterial strains from frozen pellets

By using an aseptic technique, freeze-dried bacterial pellets were re-dissolved in 1 mL TSB. One hundred µl of the culture was then plated onto TSA agar plates. The plates were inverted and incubated overnight at 37$^{o}$C. After overnight incubation, the plates were kept at 4$^{o}$C in the fridge and used as bacteria colony stock. The sensitivity of bacteria colonies was tested from time to time using commonly used antibiotics, following the MIC method described in section 3.2.6**.**

### 3.2.5 Overnight culture preparation

A few single colonies from the bacterial stock (as prepared in section **3.2.4**) were transferred to 50 ml TSB and the culture was further incubated in a water bath for at least 18 hours at 37$^{o}$C. After overnight incubation, the bacteria suspension was harvested using centrifugation at 3000 rpm for 10 minutes. The supernatant was discarded and sterile PBS (in approximately 30 ml volume) was added to the bacterial pellet. This washing step was repeated another two times using PBS, followed by centrifugation at 3000 rpm for another 10 minutes. After centrifugation, the bacterial pellets were suspended in 50ml of sterile PBS. Two ml of the

bacterial suspension was then added to a 1cm semi micro cuvette (VWR, United Kingdom) and the optical density (OD) was read at 600 nm wavelength using a Camspec M550/1 double beam scanning UV/visible spectrophotometer with PBS as the blank. The density of the bacterial suspension was adjusted to OD 0.25-0.30 (which is approximately 1-2 x $10^8$ cfu/ml) by using the PBS.

### 3.2.6 Determination of minimum inhibitory concentration (MIC)

#### *3.2.6.1 Inoculum preparation*

It is well known that inoculum size can influence MIC values (Andrews, 2001; Balouiri et al., 2016). Therefore in order to ensure consistency between the tests, the inoculum used in the MIC testing was prepared as follows: 2 mL of overnight bacterial suspension as prepared in section 3.2.5 was added to 38 mL of sterile PBS solution. This prepared suspension was used within 30 minutes of its preparation following the recommendation made by Andrews (2001).

#### *3.2.6.2 Resazurin dye preparation*

Resazurin dye is prepared by dissolving 100 mg of resazurin sodium salts (Sigma Aldrich, United Kingdom) into 100 ml RO water. The solution was heat sterilised at 121 °C for 20 minutes. The sterile resazurin solution was kept at 4$^o$C in the fridge and used within a week of its preparation.

#### *3.2.6.3 Determination of minimum inhibitory concentration*

MIC is defined as the lowest concentration needed to inhibit bacteria growth. In this study, the MIC assay was used as a tool to determine the antimicrobial status of the banana peel extract and the individual plant compounds. In general, the extracts or individual plants chemicals are considered to have 'notable antimicrobial activity' when the MIC is between 100 and 1000 µg/ml (Gibbons, 2004; Abreu et al., 2012). Using the broth dilution method, the MIC endpoints of this study were determined using resazurin dye, as proposed by Sarker et al. (2007). For MIC testing,

the extracts were prepared using 10% DMSO to a working stock of 40-50 mg/ml. For determination of the MIC of each individual compound, the standard compounds were used. The compounds were dissolved using 10% DMSO to a final concentration of 1000 µg/ml for *S. aureus* and higher concentration stocks between 1500-2000 µg/ml were used for *E. coli* MIC assay.

Briefly, 50µl of test samples (extracts/individual compound) were added to the first and second rows of the plate. Then, 50µl of sterile PBS was added to all wells except the first row. Fifty µl of the contents from the second row was then transferred to the third row and a two-fold serial dilution of the well content was carried out using multichannel pipettes. Following this, 30 µl of MHB (cation adjusted) was added to all wells, followed by 10 µl of sterile resazurin dye. Finally, 10 µl of bacteria suspension at $5x10^6$ cfu/ml concentration was added to all wells except the sterility row. Antibiotic (usually tetracycline) was used as a positive control and the wells without any samples added were used as the negative control (Sarker et al., 2007). The final arrangement for the MIC plate is given in **Figure 3-3**. In each set of MIC, columns on both sides (left and right) of the plates were used as a sterility control to ensure that each plates are free from any contamination. Blank column is used for testing the effects of the diluent used in preparing the samples to bacterial growth.

For each set of experiments, triplicate plates were prepared and incubated at 37°C for 18-24 hours. After overnight incubation, the change of colour from purple to pink was used as an indication of positive antimicrobial activity. The MIC endpoint was taken as the lowest dilution that did not display any colour change following overnight incubation.

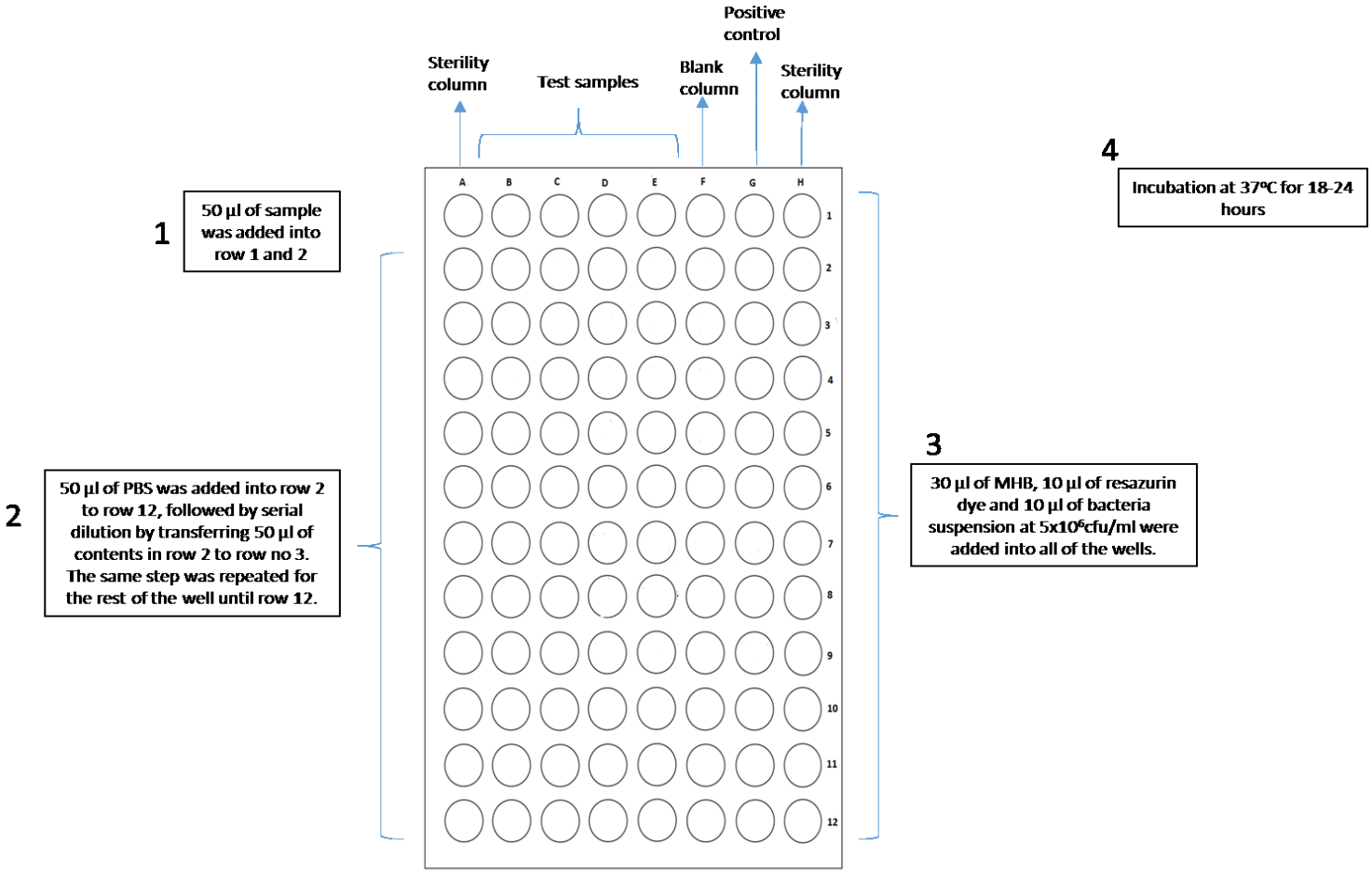



***Figure 3-3:*** *Illustrative procedure for MIC assay.*

In the sterility columns, PBS was used as a substitute to test sample and no bacteria were added into the wells. For positive control, antibiotic (i.e tetracycline) was used as the test sample. Diluent used to prepare the samples (10% DMSO) was added in the blank column.

#### *3.2.6.4 Control experiment*

In order to determine the accuracy of the MIC method, similar MIC testing as described in section 3.2.6.3 was carried out using commercial antibiotics (ciprofloxacin, tetracycline, ampicillin, amoxicillin and trimethoprim) for each bacterial species used in the study. All antibiotics used in the control experiment were prepared fresh on the day of testing (Andrews, 2001) in 100 µg/ml stock concentrations, except for ciprofloxacin where a stock at 50 µg/ml concentration was used. Due to low solubility of some of the antibiotics in water, 10% DMSO solution was used as the diluent to prepare the antibiotic stock. The MIC values recorded after 24 hours' incubation were compared with data published by Public Health England (PHE) (Andrews, 2001) as shown in **Table 3-2**.

***Table 3-2**: Target MIC (µg/ml) for reference strains.*

| **Antibiotics** | ***E. coli* NCTC 12241** | ***S. aureus* NCTC 6571** |
|---|---|---|
| **Amoxicillin** | 4 | 0.12 |
| **Ampicillin** | 4 | 0.06 |
| **Trimethoprim** | 0.25 | 0.25 |
| **Vancomycin** | - | 0.50 |
| **Erythromycin** | - | 0.12 |
| **Ciprofloxacin** | 0.015 | 0.12 |
| **Tetracycline** | 2 | 0.06 |

### 3.2.7 Water disinfection experimental protocol

Following the MIC testing, the extracts and compounds with notable antimicrobial activity were tested for their activity as a biocide against the target organism. All of the disinfection treatments were carried out in increasingly inhibitory doses. The disinfection performance was expressed in log reduction by comparing the log growth curve of the untreated cells with those challenged with test compounds.

The disinfection protocol was carried out in 250 ml Schott Duran® reagent bottles, covered using aluminium foil. All of the experiments were carried out at room temperature (±20 $^{o}$C). Aliquot amounts of overnight bacteria stock were added to 100 ml sterile PBS at a final concentration of approximately $10^6$ cfu/ml. Following this, triplicate time zero samples (1 mL) were withdrawn and serially diluted in tubes containing 9 ml sterile PBS to determine the initial concentration of the bacterial population. The test sample was then inoculated into PBS containing the bacteria solution and the timer was started. At each contact time (15, 30, 60, 120, 240 and 360 minutes), triplicate 1 ml samples were withdrawn using a sterile pipette and serially diluted ten-fold as above. The final diluted sample (10 mL) was then filtered through a 47 mm diameter sterile membrane filter with 0.45 µm pore size (PALL Corporation, New York, USA) under vacuum. Each filter membrane was then plated onto 50mm culture plates containing TSA. The plates were inverted and incubated at 37$^{o}$C for at least 18 hours. The number of surviving bacteria colonies was counted the next day using the following equation:

$$Number\ of\ bacteria\ population\ \left(\frac{cfu}{ml}\right) = \frac{colonies\ counted \times dilution\ factor}{volume\ of\ sample\ plated\ (ml)} \qquad \textbf{\textit{Equation 3-1}}$$

The control experiment was carried out without any addition of the test material, and the results from the control set were used to determine the log inhibition at 0 MIC.

#### *3.2.7.1 Control experiment*

A similar protocol to that described in section 3.2.7 was used to test the viability of each bacteria species in PBS without the addition of the extracts/test compounds. At each sampling time (15, 30, 60, 120, 240 and 360 minutes), triplicate 1 mL samples were withdrawn and diluted ten-fold with 9 ml sterile PBS. Serial dilutions (up to 5 dilutions) were performed and the sample was filtered following the membrane filtration method. The filtered sample was plated in culture plates containing TSA, and incubated at its optimum temperature for at least 18 hours. The

number of bacteria colonies was counted the next day. The results for this control experiment are presented in **Appendix C1: Quality control for *S. aureus* and *E. coli***

#### *3.2.7.2 Data analysis*

$Log_{10}$ ($N_0$/N) was used to evaluate the disinfection effectiveness, where N = number of bacteria in the treated samples after disinfection treatment (cfu/ml), and $N_0$ = the number of bacteria before treatment (cfu/ml)

Statistical analysis was performed using Microsoft Excel and Graph pad software (USA). The Student t-test was used to assess statistical differences between disinfection treatments. The null hypothesis that the log reduction was not different between different treatments was rejected at a p-value less than or equal to 0.05.

### 3.3 Assessment of the interaction between individual plant compounds

It has been reported that a combination of plant compounds could enhance or reduce the efficacy of individual compounds (Vuuren et al., 2011). Considering that extracts consist of a complex mixture of different compounds, in this study a combination of the selected compounds was tested in order to determine whether the antimicrobial activity observed in the extracts was caused by their individual compounds, or it was due to interactions between the compounds. This experiment also aimed to determine whether the combination of compounds could improve the disinfection activity of the individual compounds.

In general, combinations between antimicrobials can be evaluated using two techniques, known as the ∑FIC index factor and isobolograms methods. Both methods, however, have a similar definition of synergy, as a value of ≤0.5 is considered to be synergy; >0.5–1.0 is additive, >1.0–≤4.0 is indifferent and >4.0 is antagonistic (Vuuren et al., 2011).

### 3.3.1 ∑FIC index technique

The ΣFIC is expressed as the interaction of two agents where the concentration of each test agent in combination is expressed as a fraction of the concentration that would produce the same effect when used independently (Vuuren et al., 2011). The FIC index for each combination was calculated by using the following equation:

$$\sum FIC = \frac{MIC\ of\ compound\ A\ in\ combination}{MIC\ of\ compound\ A\ alone} + \frac{MIC\ of\ compound\ B\ in\ combination}{MIC\ of\ compound\ B\ alone} \qquad \textit{Equation 3-2}$$

Nevertheless, it should also be noted that the ∑FIC index is based on the principle that half of the concentration produces half the effect. However, this is not always the case, as two inhibitors may not always have identical dose responses. In order to overcome this limitation, the calculated ∑FIC data were also plotted using the isobologram method.

### 3.3.2 Isobologram method

The isobologram method is the oldest method used to define the combination interaction between two inhibitors. This method in general is based on the fact that interactions may differ in dose response, depending on the ratio in which the two inhibitors are combined (Vuuren et al., 2011). Using a similar equation to the ∑FIC index, the dose ratio responses of the two agents were plotted in an isobole graph. The adjoining line of the two axes indicates the individual doses and the isobologram was interpreted according to the area occupied by the data points, as shown in **Figure 3-4**.

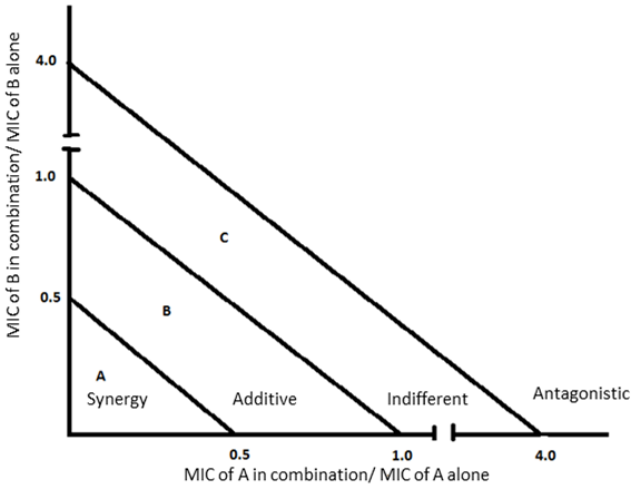



***Figure 3-4****: Isobologram interpretation; data that falls in A is considered as synergy, data in B region is considered as additive, in C region it is non-interactive and data beyond 4:4 ratio is considered as an antagonistic interaction.* Adapted from Vuuren et al. (2011)

### 3.3.3 Synergy experiment

For the synergy experiment, the checkerboard method as suggested by Berenbaum (1978) and Vuuren et al. (2011) was used. Two-fold dilutions of the compounds were prepared at a concentration of 1000 µg/ml to 0 µg/ml for GA and EGCg, and 2000 µg/ml to 0 µg/ml for catechin and epicatechin, in 2 ml micro centrifuge tubes. Next, 25µl of the two-fold dilution of compound A (the one with the lower MIC value) was added to each column of the wells, followed by the addition of 25µl of the two-fold dilution of compound B (the one with the higher MIC value) to each row of the wells. The mixture was then carefully mixed using a 50µl multichannel pipette. Similar to the MIC assay, 30 µl of broth, 10µl of resazurin dye and 10 µl of bacteria were then added to each well. The plates were incubated at 37$^{o}$C overnight. The bacteria inoculum and the MIC end point for the combination solution were determined using the same approach as in the MIC study. The ∑FIC of the compounds combination, was calculated using equation 3-2. The arrangements for synergy experiment is given in **Figure 3-5**. The combinations with synergy (∑FIC ≤ 0.5) were tested in disinfection experiment, following the protocol described in 3.2.7.

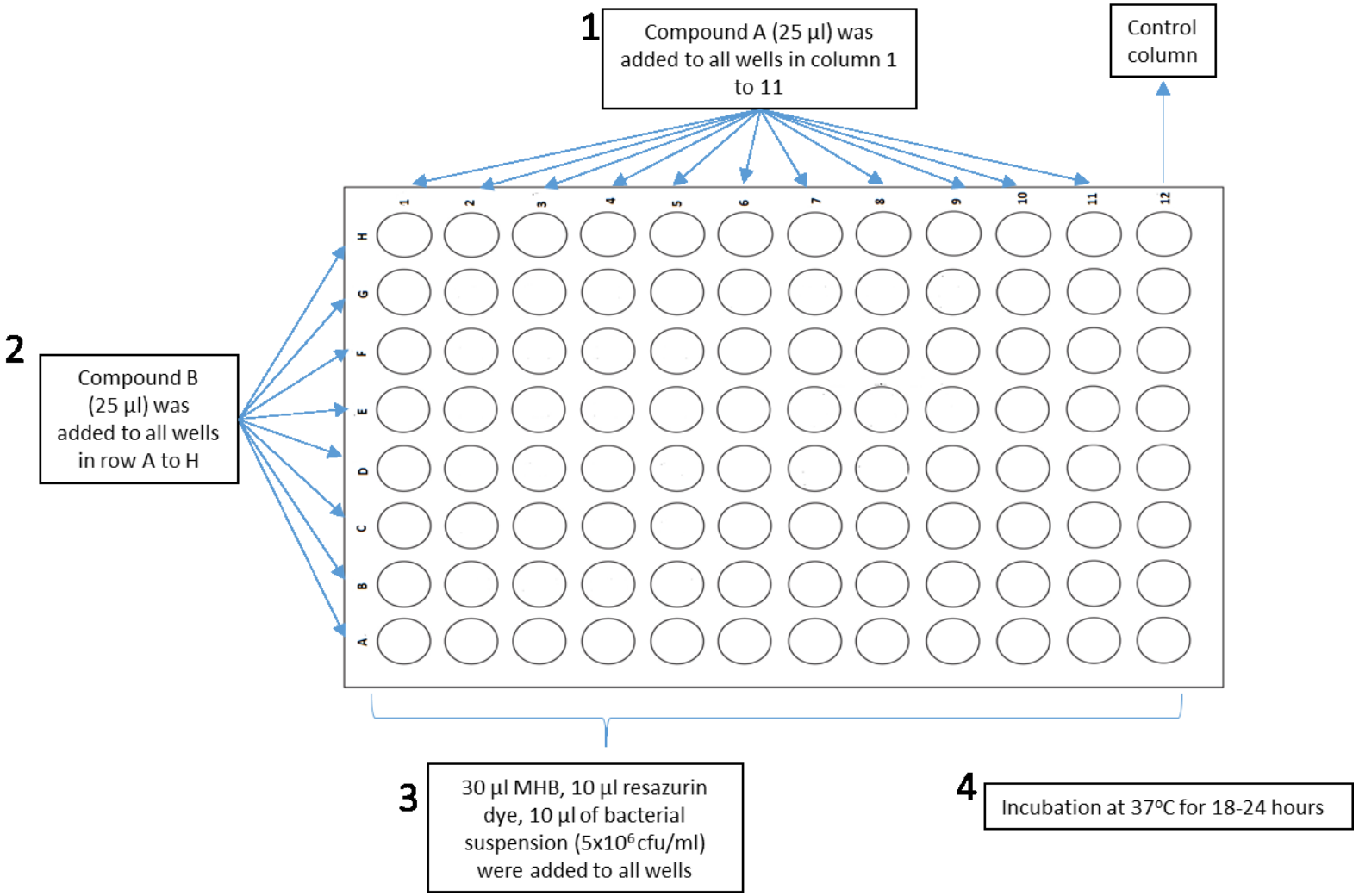



***Figure 3-5:*** *Illustrative diagram for checkerboard method.* For control column, antibiotic (i.e tetracycline) was used as the test sample.

## 3.4 Virus inactivation

### 3.4.1 Viral surrogate, bacterial host and growth medium

MS2 *coliphage* has been used as viral surrogate in many disinfection studies (e.g. Maillard et al., 1994; Sherchan et al., 2014; Sassi, 2016). In this study MS2 bacteriophage was chosen for testing the virus inactivation of the extracts and compounds, based on the fact that it possesses a morphology similar to human enteroviruses (i.e. norovirus and poliovirus) and also it is practical to use in laboratory testing (i.e. it is harmless to humans and relatively easy and quick to culture). Bacteriophage MS2 15592 and its host *E. coli* 15597-B1 were purchased from American Type Culture Collection (ATCC). The broth medium used for the *E. coli* host growth was prepared by the addition of: 10g of tryptone (Merck, Germany), 1g of yeast extract (Oxoid, Fisher Scientific) and 8g NaCl (Sigma Aldrich, USA) to 1 litre RO water, as recommended by the bacteriophage MS2 (ATCC® 15597B1™) method. The MS2 phage was enumerated using the double layer overlay method. The underlay and overlay agar were prepared by adding 15g and 4g of agar No 1 (Oxoid, Fisher Scientific) respectively to 1 litre of the above broth medium. The medium was heat sterilised in a Priorclave 230L autoclave at 121$^{o}$C for 20 minutes. Prior to the inactivation experiment, the underlay agar was prepared by plating 20 ml of heat-sterilised agar onto 90x100 mm petri dishes. The plates were left to solidify in the biosafety cabinet before they were stored in the 4$^{o}$C fridge and used within a month of production.

### 3.4.2 MS2 stock propagation

*E. coli* host (20µl) was added to 20 ml of broth and incubated overnight at 35$^{o}$C. After overnight incubation, approximately 100 µl of *MS2 coliphages* were added to the host culture. The mixture was further incubated with shaking (~100rpm) for another 16 hrs at 37$^{o}$C. The next day, the active phage suspension was separated by centrifugation at 1000 rpm for 30 minutes. The supernatant was retrieved and filtered through a 0.22 um cellulose acetate membrane (VWR, United Kingdom) to separate the remaining bacteria cell debris. The obtained suspension was

taken as MS2 stock and used within a month of production. The concentration of the MS2 stock was determined using the double agar overlay method as described in section 3.4.3 below.

### 3.4.3 MS2 stock enumeration

Prior to the MS2 enumeration, the prepared underlay agar plates were transferred to the 35$^{o}$C incubator for 1-2 hours before the experiment to dry off the remaining liquid on the agar. Nine sterile micro centrifuge tubes were filled with 900 µl of broth and labelled as 1 to 9. One hundred µl of phage stock was added to the first tube and mixed using a sterile pipette before 100 µl of the content was transferred to the second tubes. The dilution was continued until the last tube (tube no. 9), which contained approximately $1/10^9$ phage dilution. To enumerate the phage, 100 µl of each tube's contents was added to a sterile test tube containing 3 ml melted overlay agar, supplemented with 1 mM $CaCl_2$. Following this, 100 µl of the *E. coli* host was added to the test tube and the content was mixed gently before being poured onto a labelled underlay agar. The plates were allowed to solidify inside the biosafety cabinet for approximately 10 minutes before they were inverted and incubated at 37$^{o}$C for at least 18 hours. The next day, the clearing area (plaques) in the lawn of the confluent *E. coli* host was counted. The MS2 titer was determined using the following equation:

$$MS2\ titer\ \left(\frac{pfu}{ml}\right) = \frac{plaques\ counted \times dilution\ factor}{volume\ of\ sample\ plated(ml)} \qquad \textbf{\textit{Equation 3-3}}$$

### 3.4.4 Virus inactivation protocol

Aliquot amounts of virus stock were added to a series of test tubes containing 5ml sterile PBS solution to a final concentration of $10^5$ plaque-forming units (pfu)/ml. Then, 100 µl of the content was withdrawn and serially diluted in 900µl broth to determine the initial concentration of the phage in each set of the experiment. The test sample was then added to the tubes and the timer was started. For sampling, triplicate samples (100µl) were withdrawn after 10, 20, 30, 40 and 60 minutes' incubation. Samples were serially diluted in 1.5ml micro centrifuge tubes containing 900µl broth solution to a desired dilution that would produce approximately 30-300

plaques per plate. Next, the diluted samples were added to 3ml overlay agar containing 100 µl of the *E. coli* host. The mixture was mixed and poured onto prepared underlay agar plates. After the agar had solidified, the plates were inverted and incubated at 37°C overnight. The number of MS2 titers was counted the next day using equation 3-3. Tubes with the addition of sterile PBS were used as an untreated control. Each inactivation trial was conducted under identical conditions in three independent replicates.

#### *3.4.4.1 Data analysis*

$Log_{10}$ ($N_0$/N) was used to evaluate the disinfection effectiveness, where N = number of virus titer in the treated samples after disinfection treatment (pfu/ml), and $N_0$ = the number of virus titer before disinfection (pfu/ml)

Statistical analysis was performed using Microsoft Excel and Graph pad software (USA). The Student t-test was used to assess statistical differences between disinfection treatments. The null hypothesis that the log reduction was not different between different treatments was rejected at a p-value less than or equal to 0.05.

### 3.5 Hydrogen peroxide generation

One of the proposed modes of action for plant-derived compounds occurs through the generation of hydrogen peroxide (Arakawa et al., 2004;Kajiya et al., 2001; Nakayama et al., 2015). In order to confirm this hypothesis, the amounts of endogenous hydrogen peroxide generated by the test samples (extracts/ individual compounds) were measured using FOX assay, as recommended by many studies involving biological samples (Nourooz-Zadeh et al., 1995; DeLong et al., 2002; Li et al., 2017). The FOX measurement requires a standard calibration curve of a known concentration hydrogen peroxide solution. Therefore, section 3.5.1 is used to describe the methods for the preparation of the permanganate solution used to determine the concentration of the hydrogen peroxide stock solution, and section 3.5.2 is used to describe the steps involved in quantifying the concentration of the stock hydrogen peroxide solution through permanganate

titration. A known concentration of hydrogen peroxide solution obtained through the permanganate titration was then used to generate the calibration curve in the FOX experiment.

### 3.5.1 Standardisation of potassium permanganate solution

Since potassium permanganate is a strong oxidising agent, the molecules of which can easily decompose over time, the concentration of the prepared permanganate solution needs to be determined using oxalic acid titration. In this method, the reduction reaction of permanganate by the oxalate was carried out in acidic conditions.

Oxalic acid solution in 0.1 N was prepared by dissolving 4.51 g oxalic acid (Fisher Scientific, United Kingdom) in 1 litre RO water. Sulphuric acid in 2 N concentration, used as a reaction catalyse, was prepared by adding 96% concentrated acid stock (Fisher Scientific, United Kingdom) to 55.62 ml of RO water. The potassium permanganate solution was prepared at approximately 0.1 N concentration, by adding 3.2 g permanganate powder (BDH Laboratory Supplies, England) and 500 ml RO water to a 1-litre beaker. The permanganate solution was boiled and stirred at 100 rpm for 1 hour. Afterwards, the solution was cooled down to room temperature and transferred to 1 litre amber colour volumetric flask. The volume of the permanganate solution was adjusted to 1 litre by adding RO water to the flask. The permanganate solution was kept at 4$^{o}$C in the fridge until used.

For titration, the permanganate solution was transferred to a 50 ml burette and the initial volume was recorded. Using a clean 250 ml flask, 10 ml sulphuric acid solution was mixed with an equal amount of oxalic acid. The acid solution was heated to 60-80$^{o}$C, after which the permanganate solution was slowly titrated into the acid mixture until a light rose colour appeared. Since the oxalate-permanganate redox reaction reacts slowly at room temperature, the acid solution was kept hot (temperature $\geq$ 70$^{o}$C) during the titration. The experiment was repeated at least 5 times before the average normality of permanganate solution was determined.

Using a ratio of 5 moles $C_2O_4^{2-}$/ 2 moles of $MnO_4^-$, the moles of oxalate were converted into moles of permanganate to determine the concentration of the permanganate solution. Full

raw data on permanganate titration is provided in **Appendix F1: Raw data from Potassium permanganate titration**.

### 3.5.2 Determination of hydrogen peroxide stock concentration

For hydrogen peroxide determination, a concentrated sulphuric acid solution was prepared by adding 50 ml of 96% grade sulphuric acid (Fisher Scientific, United Kingdom) to 150 ml of RO water in a 250 ml beaker. The solution was allowed to cool to room temperature before being used. Five grams of hydrogen peroxide (35% w/w) solution (Acros Organic, Belgium) was weighed and added to a 10 ml beaker. The weight gain of the beaker after the addition of hydrogen peroxide was recorded as W. The hydrogen peroxide solution was then transferred to a 500 ml volumetric flask, followed by the addition of 250 ml RO water and 2 ml of sulphuric acid solution. The beaker was thoroughly rinsed using RO water and the contents were diluted to the 500 ml mark using RO water. For titration, 20 ml of the solution was transferred to a 250 ml flask containing 15ml sulphuric acid and 60 ml RO water. Using the same potassium permanganate solution prepared in section 3.5.1, the titration was carried out using a 50 ml burette until the light pink colour persisted for approximately 30 seconds. The permanganate volume was recorded as V. The procedure was repeated at least five times and the average volume of the permanganate used was calculated. Finally, the concentration of the hydrogen peroxide stock solution was determined using the following equation:

*Percentage of hydrogen peroxide (w/w) = (V) (N) (1.701) (25)/ (W)* ***Equation 3-4***

- V volume of titrated permanganate
- N normality of the permanganate
- W grams of $H_2O_2$ weight in 500ml volumetric flask
- 1.701 weight per miliequivalent of $H_2O_2$ x 100
- 25 dilution factor

### 3.5.3 Ferrous oxidation-xylenol orange (FOX) assay

A FOX assay is based on the oxidation of ferrous ions to ferric ions by hydrogen peroxide, which binds with xylenol orange to form a complex product. The formation of this complex can be measured using a UV spectrometer at 560nm wavelength (Bou et al., 2008). The FOX assay has been used previously to quantify the amount of hydrogen peroxide in honey (Li et al., 2017), edible oils (Nourooz-Zadeh et al., 1995) and plant tissue (DeLong et al., 2002).

In this study, the FOX assay was carried out following the method by DeLong et al.(2002). The protocol of the assay is as follow: the working FOX reagent was freshly prepared on the day of the experiment by adding 9 parts of reagent 2 to 1 part of reagent 1. Reagent 1 was prepared by adding 98.03 mg of ammonium ferrous sulphate (Fisher Scientific, United Kingdom) to 250 mM sulphuric acid ($H_2SO_4$) (Fisher Scientific, United Kingdom). The solution was mixed until the complete dissolution of the ammonium ferrous sulphate was achieved. Following this, 76.06 mg of xylenol orange (Sigma Aldrich, United Kingdom) was added to the acidic ammonium ferrous solution. Reagent 2 was prepared by dissolving 969.76 mg of butylated hydroxytouene (BHT) (Sigma Aldrich, United Kingdom) in 900 ml of HPLC-grade methanol. The final working FOX solution consisted of 100 μM xylenol orange, 4.4 mM BHT, 25mM sulphuric acid and 250μM ammonium ferrous sulphate. The prepared FOX reagent was calibrated using varying concentrations of hydrogen peroxide solution at 560 nm wavelength using UV-2401 PC Spectrophotometer (Shimadzu Corporation, Japan).

A typical calibration curve used in the quantification of the samples' hydrogen peroxide is demonstrated in **Figure 3-6**. The adherence of the method to Beer's Law was obeyed when the concentration of hydrogen peroxide used was in the range of 0-11.25 μM before it started to plateau, as observed in **Figure 3-7** below. Therefore, during hydrogen peroxide quantification, the limit of detection of this FOX assay was set at 11.25 μM.

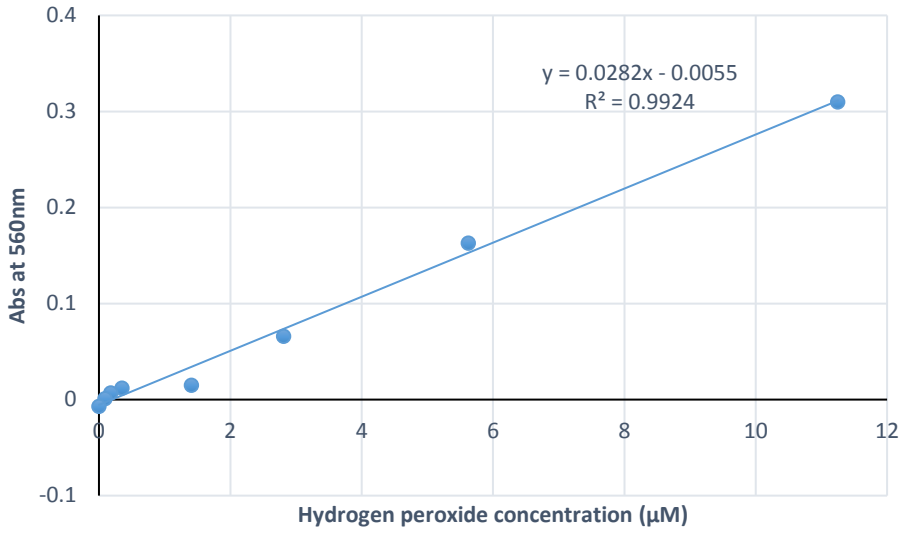



***Figure 3-6**: Calibration curve of hydrogen peroxide using FOX assay, measured at 560nm*

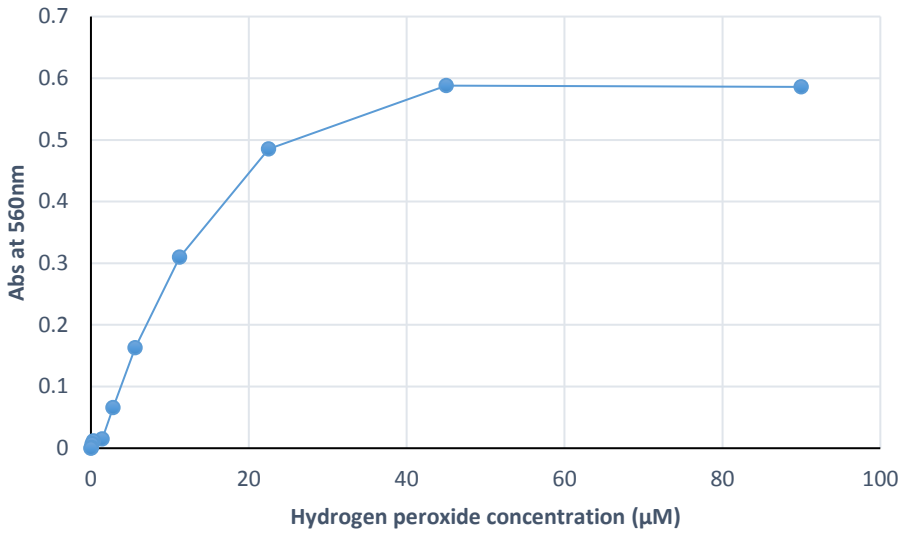



***Figure 3-7**: Hydrogen peroxide calibration from 90µM to 0µM*

### 3.5.4 Quantification of hydrogen peroxide generated from samples

For hydrogen peroxide measurement in the samples, a similar experimental procedure as in the water disinfection was followed, except there was no bacteria added into the PBS solution. At each sampling time (30, 60, 120, 240 and 360 minutes), aliquot amounts of sample was withdrawn, and 180 µl of sample was added to a 2ml micro centrifuge tube. Subsequently, 20µl of HPLC-grade methanol was added to the tube, followed by 5s agitation using a Vortex Genie 2. The sample was then incubated for 30 minutes. After the incubation, 1800 µl of FOX reagent was added to the methanol solubilised sample. The mixture was incubated for another 30 minutes at room temperature under dark conditions. Finally, the mixture was centrifuged for 10 minutes. The absorbance of the sample was read at 560nm wavelength, and compared with the standard curve to obtain the concentration of hydrogen peroxide in the sample.

# Chapter 4 RESULTS

## 4.1 Production of the extracts and compound identification

Banana peels have been shown to contain significant amounts of phenolic compounds (Someya et al., 2002; Mokbel and Hashinaga, 2005; Rebello et al., 2014). Therefore, in the first part of this study, the results from the extraction procedure will be presented. It is known that factors such as solvents, solute and solvent ratio, and temperature play an important part in any extraction procedure. Nonetheless, comprehensive studies on optimised extraction conditions for banana peel extraction yields have been carried out previously only by González-Montelongo et al. (2010) using the same species of banana (Musa Cavendish) as in this study. The effects of solvents, temperature and pH on extract yield were examined. The results of their study suggested that methanol is the most effective solvent for extracting phenolics in banana peel, resulting in higher biological activity (i.e. antioxidant activity as in their study). Therefore, in this study, methanol was chosen as the extractant for obtaining the crude banana extracts. In addition, at the beginning of this study, an attempt to fractionate the crude extracts into several fractions using the non-polar solvents hexane and chloroform were carried out. The results showed that, fractionation using non-polar solvents, i.e. hexane and chloroform, failed to produce extracts with antimicrobial activity, with no compounds extracted from crude banana peels (**Appendix A2: Fractionation of methanolic extracts with hexane and chloroform**). This result showed that banana peel extracts in general consist of polar compounds; thus comparative data on the effects of different polar solvents (methanol, ethanol, acetone and water) on banana peel-derived bioactive compounds, as presented by González-Montelongo et al. (2010), are sufficient to obtain optimised banana peel extracts.

It has also been proposed that temperature could improve the extraction performance, as high temperatures have been reported to improve the yield products. Such factors, however, cannot be considered for plant extraction studies as plants' phenolic compounds tend to degrade at temperatures above 40$^{o}$C (Sarker and Nahar, 2009). Considering that, all of the extraction

procedure was carried out at room temperature. Nevertheless, it has been shown that basic processing steps such as drying have a significant impact on the rate of solubilisation of phenolic compounds in plant materials (Tsami et al., 1998; Vuong et al., 2015; Thi and Hwang, 2016) such as bananas. Therefore, in this study, the effects of different dried banana peels were tested simultaneously with extraction time.

Drying plays an important role in plant material processing and it is recommended by many researchers as it reduces the material moisture content, thus preventing the growth of moulds and further enzymatic reaction to the materials. Phenolic compounds in general are susceptible to enzymatic activity, known as polyphenols oxidase activity, which results in browning effects in fruits and vegetables. This enzymatic activity has also been shown to contribute to the degradation and reduced quality of the material (Sanchez-maldonado, 2014). Therefore, in this study, two types of drying systems were considered: oven drying and freeze drying. The effects of drying on extraction efficiency were observed by comparing the amounts of extractable solids (yield) obtained after the evaporation of the solvents to dryness.

As illustrated in **Figure 4-1**, higher product yields were observed in banana powders prepared through freeze drying compared to those prepared with oven drying ($p<0.05$). Freeze-dried material is known to have a more porous structure, with little or no shrinkage compared to oven-dried material (Lee et al., 2012; Chung and Lee, 2015), therefore, it is likely that better solubilisation between the solute and solvents can be achieved in freeze-dried banana peel powder. The results regarding solvent recovery (**Figure 4-2**) also indicate that extraction using freeze-dried banana peels provides a higher degree of rehydration to the material, as lower amounts of solvent were recovered from freeze-dried material compared to the oven-dried. This observation may also suggest that better solubilisation of naturally occurring substances was achieved in freeze-dried materials, thereby providing higher amounts of active metabolites that could be used as a biocide. Compared to freeze-dried banana peels, extraction using oven-dried peels was shown to be less effective in extracting compounds from banana peels, with the

minimum yield being observed during a 1-hour maceration period ($p<0.05$, when compared to freeze-dried banana peels).

As observed, the production of extracts in freeze-dried banana peel varies with an increase in extraction duration, though the effect is not statistically significant between extraction times ($p>0.05$). The results in this study are in contrast with other studies which reported that an increase in product yield was achieved when a longer extraction time was utilised (González-Montelongo et al., 2010). No obvious trend between extraction time and the yield produced was found in oven-dried banana extracts – inconsistent product yield was observed when the maceration was extended from 1 hour to 48 hours. Overall, the findings indicate that banana peels produced through freeze drying have higher quality in terms of their extraction yield and also in their antimicrobial activities, which will be discussed in a later section (4.2). Therefore, the freeze-dried banana peel was chosen for further study in the disinfection assessment.

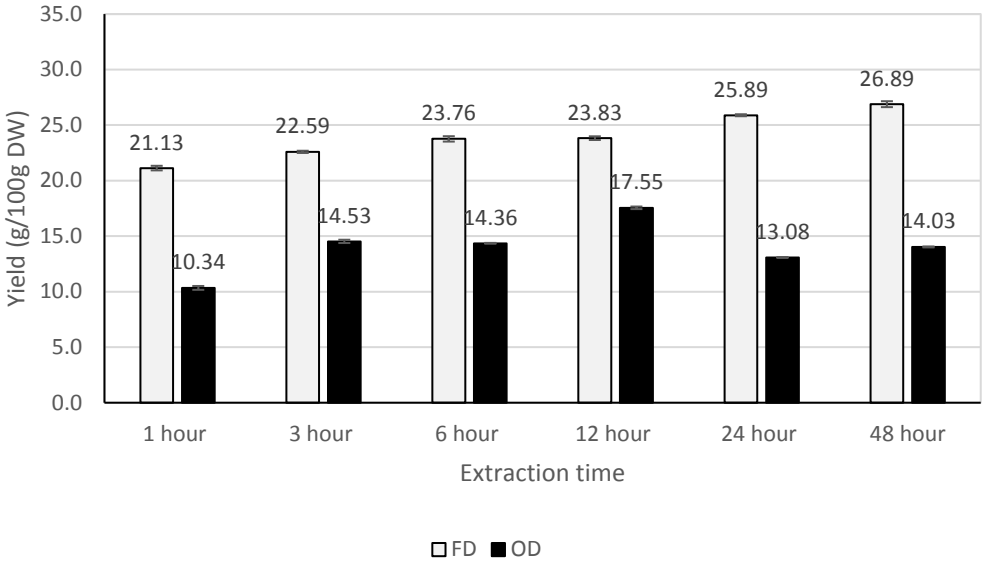



***Figure 4-1:*** *The yield of banana peel extracts at different extraction times.* DW: dry weight of banana peels; FD: freeze-dried banana peel extracts; OD: oven-dried banana peel extracts. Results shown are representative of three independent experiments. Drying the peels using freeze drying improved the extracts yield compared to oven drying ($p<0.05$).

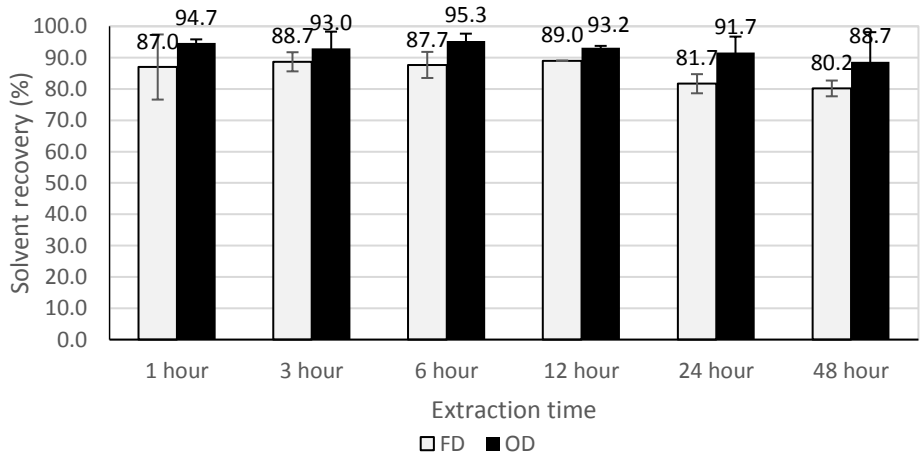
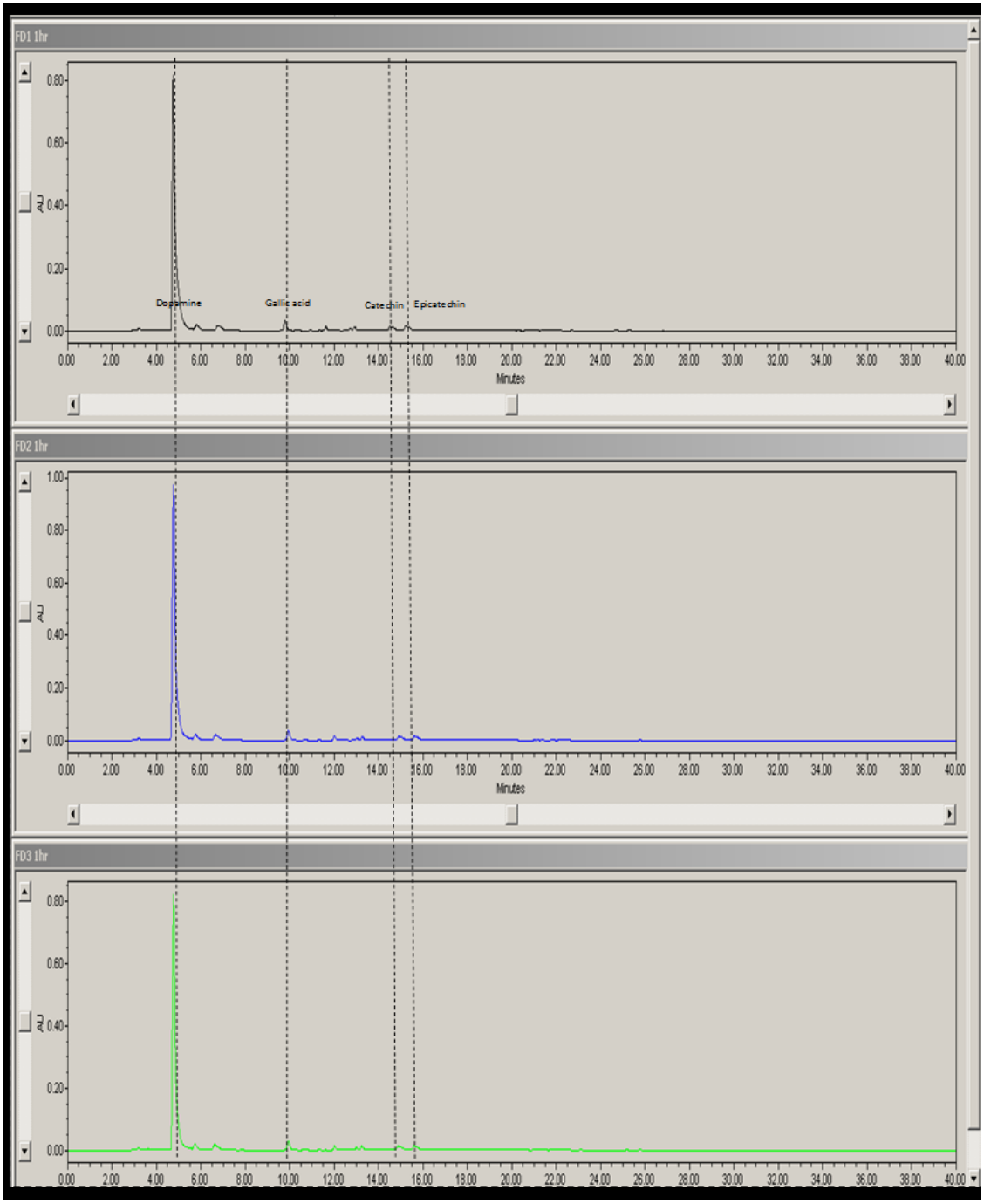


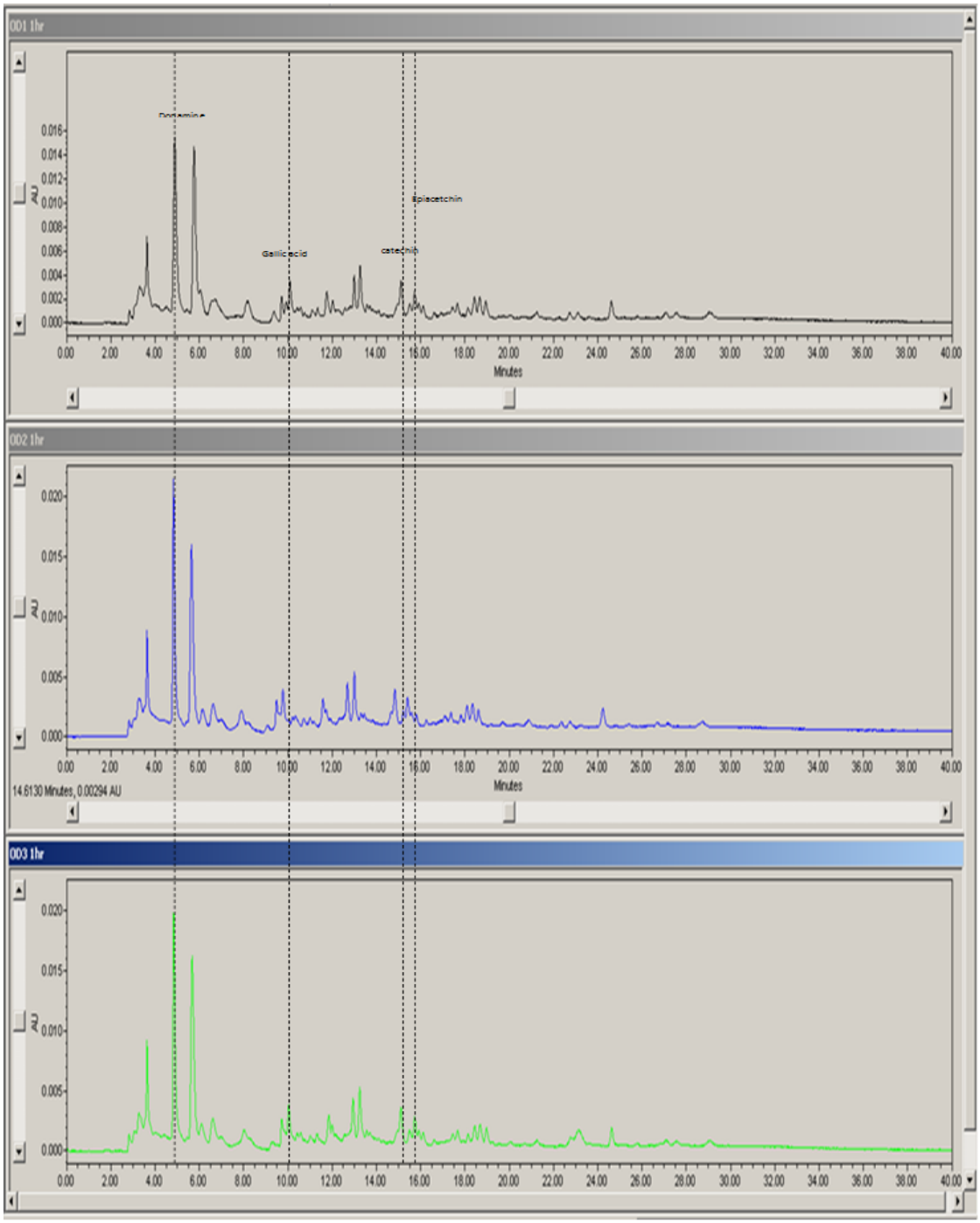

***Figure 4-2:*** *The percentage of solvent recovered at different extraction times, for both freeze dried and oven-dried banana peels.* FD: freeze-dried banana peel extracts; OD: oven-dried banana peel extracts. Results shown are representative of three independent experiments.

### 4.1.1 HPLC fingerprint of the extracts

As it is known that extracts consist of a complex mixture of compounds, a fingerprinting study was carried out to better understand the chemical composition of the extracts and examine the consistency of the extraction procedure. Chromatographic fingerprinting is a qualitative method used to characterise the profile of extracts, as recommended by the US Food and Drug Administration (FDA), the European Medicines Evaluation Agency (EMEA) (Li et al., 2015). For the fingerprinting study, the extracts obtained from each extraction were dissolved in 20 mL of methanol, followed by the injection of the samples to the HPLC. Examples of the chromatograms for oven-dried and freeze-dried peel extracts are given in **Figure 4-3**. A full database on fingerprinting can be found in the **Appendix A3: Chromatograms for the banana peel extract fingerprinting study**. As observed, drying the peel using freeze-drying and oven-drying completely changed the phenolic profile of the extracts, with more compounds present in low quantities in oven-dried extracts. This is likely due to the degradation of the main bioactive compounds into smaller molecules in the oven-dried extracts. The phenolic profile for freeze-dried extracts obtained from this study is similar to those produced by Del Mar Verde Mendez et al. (2003) using a fresh banana sample. Overall, both extracts (oven dried and freeze-dried) produced almost identical phenolic profiles and there was consistent compound elution between the replicates, which allowed confidence in the reproducibility of batch-to-batch banana peel extracts produced in this study. The dotted lines represent the position of four marker compounds identified in section 4.1.2. However, due to low antimicrobial activity exhibited by the oven-dried extracts, only the compounds in the freeze-dried banana extracts were quantified. As illustrated in the chromatograms, extracts from freeze-dried peels were higher in phenolic content (higher in absorbance value) compared to the extracts of oven-dried banana peels, suggesting that freeze-drying is more practical for processing banana peels as antimicrobial agents.


FD1 1hr
Dopamine
Gallic acid
Cate chin
Epicate chin
AU
Minutes
FD2 1hr
AU
Minutes
FD3 1hr
AU

(a)




(b)

***Figure 4-3:*** *Chromatograms of (a) freeze-dried (b) oven-dried banana peels, extracted at 1 hour maceration time.* Chromatograms shown for three independent extractions. The dotted lines represent the identified compounds, from left to right: Dopamine, Gallic acid, Catechin and Epicatechin.

### 4.1.2 Chemical composition of freeze-dried banana extracts

Due to the low level of activity exhibited by the oven-dried banana extracts (**Figure 4-6** and **Figure 4-7**) on two of the bacterial species, only freeze-dried peel extracts were quantified using HPLC. Four main compounds – dopamine, gallic acid (GA), catechin (C) and epicatechin (EC) – were identified in the freeze-dried banana peels (**Figure 4-4**). Consistent with the results, Del Mar Verde Mendez et al. (2003) also identified the presence of catechin and gallic acid in their banana sample from Ecuador. The identification of the compounds in the extracts was made by comparing retention times and the injection of internal standard into the extracts. Supplementary data for the standard compounds is provided in **Appendix A4: Supplementary data for HPLC procedures**. The quantitative analysis of the identified compounds from different maceration times was quantified using a standard calibration curve from commercially available standards. The details for compound calibration are given in **Table 4-1**.

Among all of the compounds identified in the extracts, only gallic acid, catechin and epicatechin were recognised as having antimicrobial activity (Matsunaga et al., 2010; Borges et al., 2013; Luís et al., 2014; Gopal et al., 2016); therefore, these compounds were chosen for further investigation in the antimicrobial and antiviral studies in this research. Dopamine in general is known to be a neurotransmitter, and the absence of dopamine has been linked to Parkinson's Disease (Kanazawa and Sakakibara, 2000). Previous antimicrobial studies on dopamine ester have been carried out by Sellami et al. (2013) on different species of Gram-negative and Gram-positive bacteria. Nevertheless, dopamine on its own has been shown to be inactive against bacterial species (Sellami et al., 2013), therefore was not considered further in this research as a likely antimicrobial agent.

The composition of the identified compounds is presented in **Figure 4-5**. Increases in the extraction time (i.e. from 1 hour to 3 hour) did not statistically affect the amount of identified compounds extracted from the freeze dried banana peels ($p>0.05$). Although an increase in extraction time was shown to be effective in achieving a more extractable yield, as observed in **Figure 4-1**, a reduction in total phenolic composition was noted when the extraction was carried

out for longer. This is likely to be due to the degradation or oxidation of phenolic compounds, as mentioned by Hertog et al. (1992). Indeed, the lowest compound composition was noted from 48-hour extracts, with a total of approximately 180 mg phenolics/100 g DW.

As shown in **Figure 4-5**, catechin was the most abundant compound extracted from freeze-dried banana peel extracts. At 1 hour maceration time, more than 300 mg/100 g DW of catechin was extracted, and its concentration increased to more than 400 mg/100 g DW when the extraction was continued for 3 hours. Increasing the extraction time beyond this, up to 24 hours, was, however, not effective in extracting more catechin ($p>0.05$) from the banana peel sample and at 48 hours' extraction, the concentration of catechin was actually significantly reduced ($p<0.05$). Epicatechin was the second highest compound found in the freeze-dried banana extracts. Compared to catechin, increase in extraction time, did not statistically ($p>0.05$) affect the amounts of epicatechin extracted from the freeze dried banana peels, suggesting that extraction time had no effect on epicatechin composition in the extracts. It is known that epistructured catechins (such as EC and EGC) are prone to epimerisation reactions with increased extraction times, which convert them into non-epistructured catechins (Saklar et al., 2015). This therefore explains higher catechin concentration compared to epicatechin in all maceration time.

Similar to catechin, a higher concentration of gallic acid was observed during the first 6 hours' maceration, before it was reduced significantly to 10.47 mg/100 g of DW after 48 hours maceration ($p<0.05$). The concentration of gallic acid in the banana peels extracts obtained during the first 6 hours' maceration in this study was slightly higher compared to the 29 mg/100 g DW in banana pulp as reported by Pereira and Maraschin (2015); this is also consistent with an earlier study that suggested banana peels have higher bioactive compounds compared to their edible part (Someya et al., 2002).

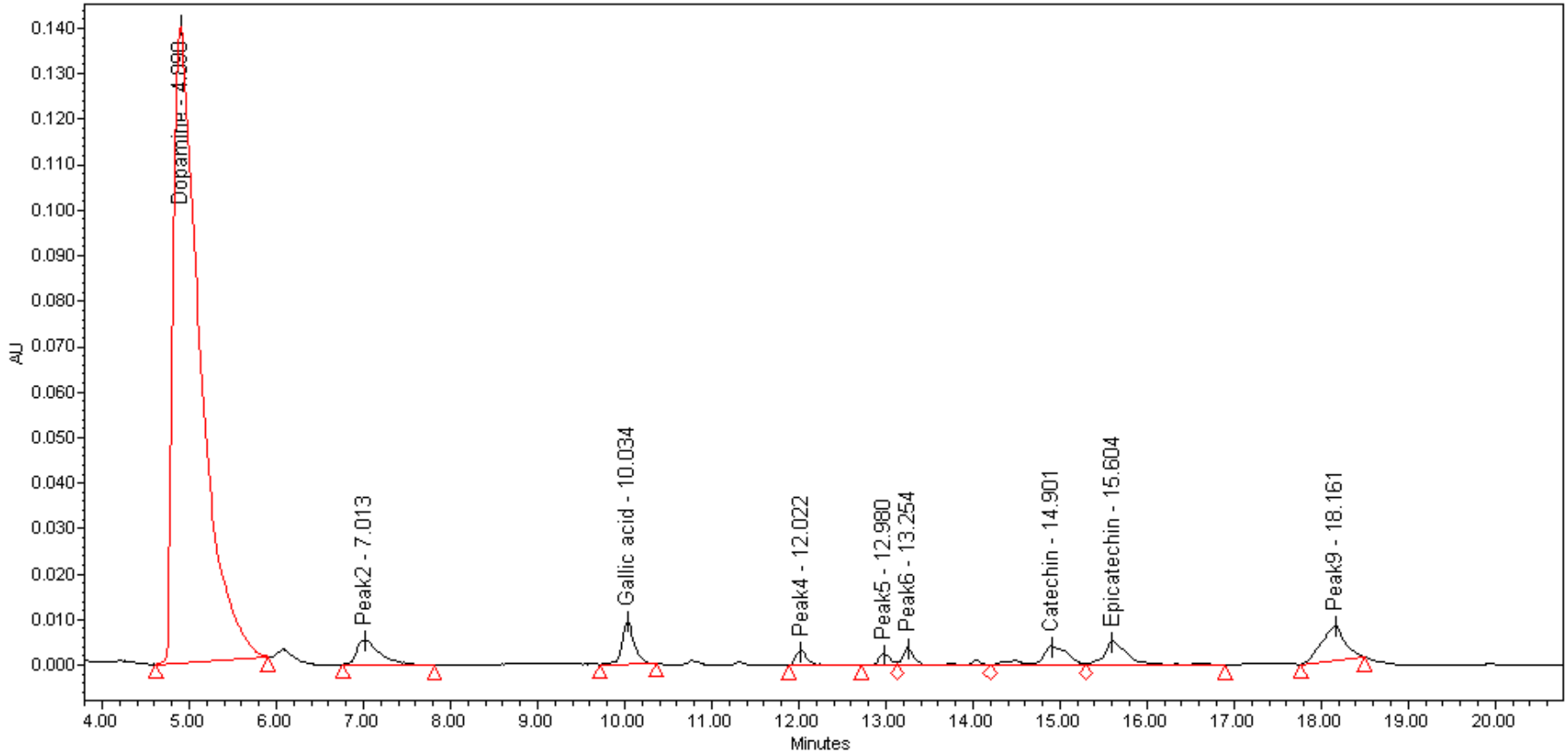



***Figure 4-4**: Elution time for compounds identified in freeze-dried banana peel extracts.*

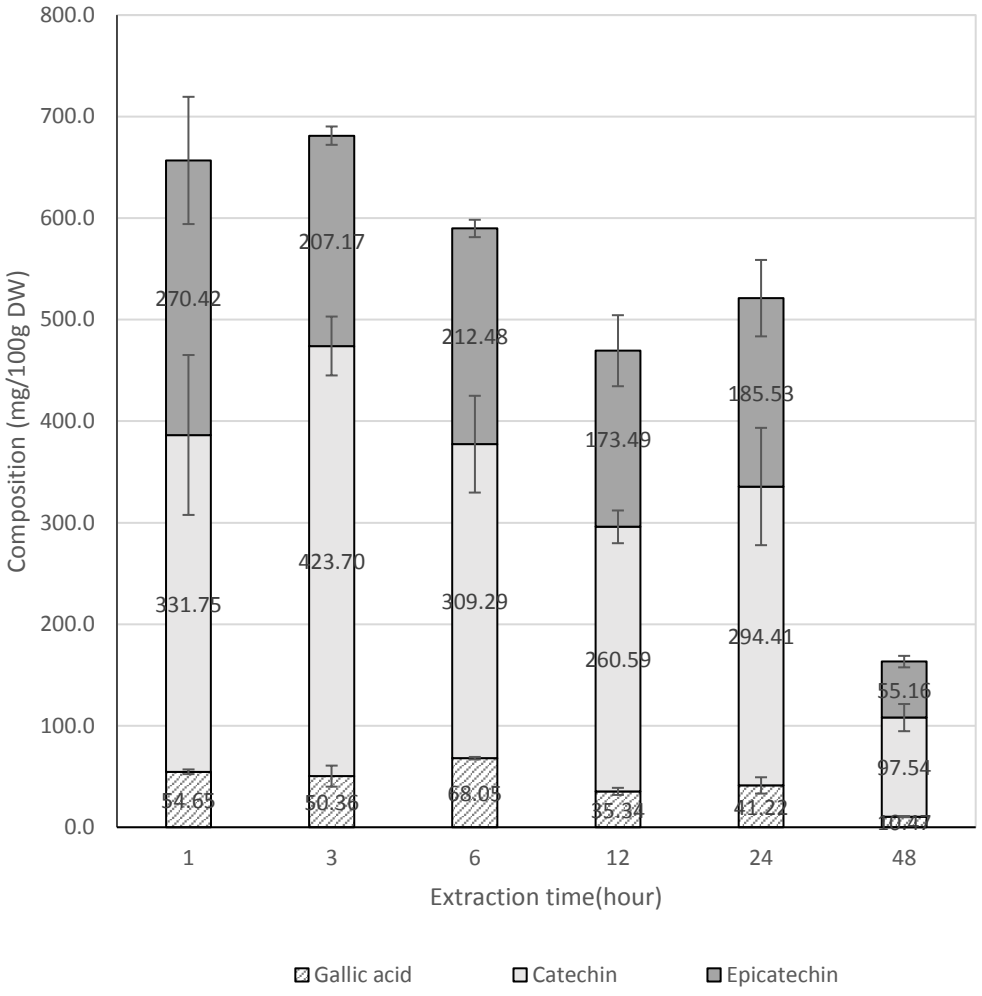



***Figure 4-5:*** *Composition of gallic acid, catechin and epicatechin in freeze-dried banana extracts at different extraction times.* The results shown are representative of three independent samples (mean ± standard deviation).

***Table 4-1:*** *Calibration range, regression coefficients, and limit of detection of the identified banana extract compounds*

| Compound | Concentration range (µg/ml) | Regression line | $R^2$ | LOD (µg/ml) | Recovery rate (%) |
|---|---|---|---|---|---|
| **Dopamine** | Not determined | | | | |
| **Gallic acid** | 0.313-10 | y = 137961x + 11006 | 0.9993 | 0.313 | 72.11 |
| **Catechin** | 0.625-10 | y = 23463x - 2751 | 0.9997 | 0.625 | 103.31 |
| **Epicatechin** | 0.313-10 | y = 29296x + 3798.4 | 0.9962 | 0.313 | 72.57 |

## 4.2 Antimicrobial activity of banana extracts and their individual compounds

### 4.2.1 Determination of minimum inhibitory concentration of the banana extracts

The antimicrobial activity of phenolic compounds can be determined by measuring their minimum inhibitory concentration (MIC). Phytochemicals in general are considered as antimicrobials if the recorded MIC is between 0.1 and 1.0 mg/ml (Gibbons, 2004; Abreu et al., 2012). In this study, the antimicrobial activity of banana peel extracts was tested on two different bacteria species, namely *S. aureus* NCTC 6571 and *E. coli* NCTC 12241. The selection of bacteria strains was based on the recommendations for control strains made by Public Health England (PHE, 2018). In order to ensure the accuracy of the method, the bacterial strains were also tested on commonly used antibiotics, and the MICs of each antibiotic were compared with available data provided by Andrews (2001). The results for the antibiotic assessment on both bacterial strains are presented in the **Appendix B3: Quality control using commercial antibiotics for MIC**. In general, it can be seen that the MIC data on both of the bacterial species when tested with antibiotics agrees with the published MIC data, indicating that the bacteria and method used in this study followed the standards set by CLSI (CLSI, 2012). The addition of resazurin as an indicator of bacterial growth did not compromise the endpoint of the MIC data point.

A previous study that conducted banana extract MIC assays was carried out using extracts dissolved in methanol (Mokbel and Hashinaga, 2005); however, it should also be noted that methanol could exhibit bactericidal activity due to its partial lipophilicity character on bacteria (Thuille et al., 2003). Therefore, in this study, the extracts were dissolved in 10% DMSO before the MIC test was carried out. The 10% DMSO did not show any inhibition on any of the bacteria tested (**Appendix B1: MIC experiment culture plate**). **Figure 4-6** and **Figure 4-7** show the MIC values of both banana peel extracts (freeze dried and oven dried) for *S. aureus* and *E. coli* bacteria. As can be observed, freeze-dried banana peels are more active (i.e. lower MIC value) towards both bacterial species, compared to oven-dried banana extracts. This result therefore confirmed earlier assumption made in section 4.1 that drying the peels using freeze drying

methods is more effective in extracting active metabolites from banana peels, thus producing extracts with higher antimicrobial activity. The results also indicate that *S. aureus* bacteria in general are more sensitive than *E. coli* to banana peel extracts (freeze dried and oven dried). The higher antimicrobial activity observed in *S. aureus* compared to *E. coli* is in agreement with earlier study by Mokbel and Hashinaga (2005) who tested the activity of ethyl acetate (EtOAc) fraction of green banana peel extracts using disk diffusion method.

Among all the freeze-dried banana extracts tested, only the extracts produced within 3 hours' extraction can be considered as promising antimicrobials for both bacteria species, though a slightly higher quantity of the extracts is required to inhibit *E. coli* growth. The recorded MICs of banana peel extracts at 3 hours' extraction were 0.63 mg/ml and 1.25 mg/ml or *S. aureus* and *E. coli*, respectively. As shown in **Figure 4-6**, freeze-dried banana extracts obtained beyond 6 hours' extraction time were found to be less active towards both bacteria tested, suggesting that longer extraction may contribute to the degradation of active antimicrobial compounds. This was true as the lowest activity was observed during 48 hours' maceration, and a higher quantity of the extract was required for the inhibition to take place. In addition, extracts from 1, 3 and 6 hours' maceration exhibited similar activity towards *S. aureus* with an MIC value of 0.63 mg/ml, which may possibly be due to similarities in their composition, as observed in **Figure 4-5**.

It is an interesting observation that, between the compositions of polyphenol compounds shown in **Figure 4-5**, and the MIC of freeze-dried extracts obtained through different maceration times shown in **Figure 4-6**, that the MICs of the extracts were linked to the total active antimicrobials available in the extracts. However, whether the resulting antimicrobial activity was due to individual compounds or combination activity of the compounds, will be covered later in this chapter. Nevertheless, studies on polyphenol compounds have shown that catechins (the dominant compounds in the extracts) in particular have a higher susceptibility to Gram-positive than to Gram-negative bacteria (Kajiya et al., 2001; Nakayama et al., 2013; Yoda et al., 2004), which may explain the high susceptibility of the extracts towards *S. aureus* compared to *E. coli*.

It should be emphasised that it is beyond the scope of this study to isolate each compound, as compound isolation usually results in a low extractable yield of individual compounds (sometimes in micrograms), which is not feasible for the disinfection assessment to be carried out. Therefore, for the evaluation of individual compounds, commercially obtained standard compounds were used instead, as they are known to have more consistent purity than isolated compounds. As such, the antimicrobial activity of the individual standard compounds was determined, and the results are presented in **Figure 4-8**.

The antimicrobial effects of identified banana peel compounds were tested in parallel with epigallocatechin gallate (EGCg), a widely studied catechin compound, to assess their inhibitory activity as a biocide. EGCg in general is a by-product of gallic acid and catechins' esterification. Higher quantities of EGCg, together with EC and gallic acid, have been reported in green teas (Taylor et al., 2009). Furthermore, the presence of EGCg in banana extracts also has been reported recently (Septembre-Malaterre et al., 2016).

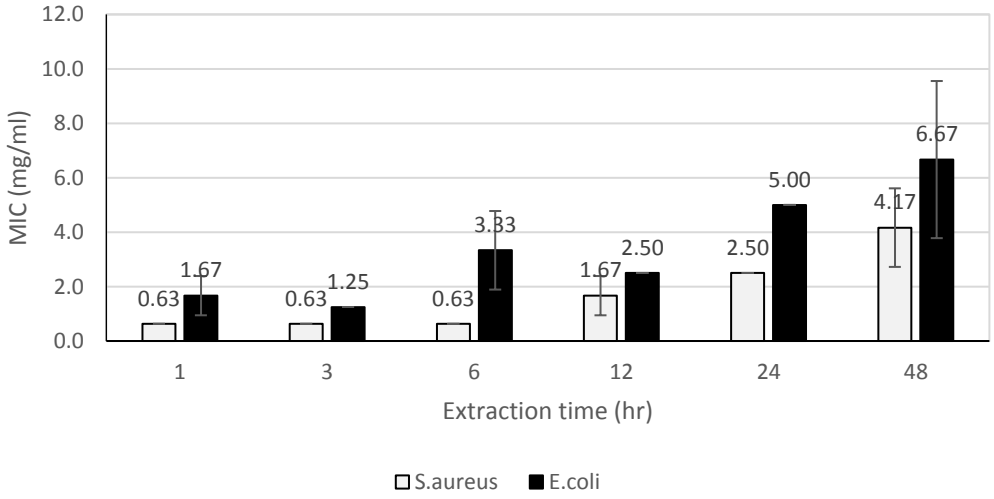



***Figure 4-6:*** *Minimum inhibitory concentration (mg/ml) of freeze-dried banana peel extracts towards S. aureus (NCTC 6571) and E. coli (NCTC 12241).* The results were determined using broth microdilution after overnight incubation at initial population of $5x10^5$ cfu/ml. The results shown are mean ± standard deviation of three independent replicates.

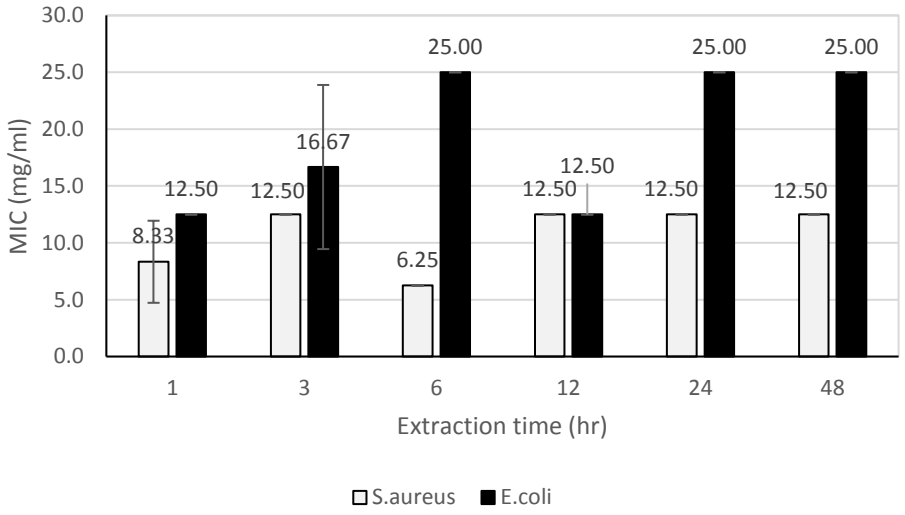



***Figure 4-7:*** *Minimum inhibitory concentration (mg/ml) of oven-dried banana peel extracts towards S. aureus (NCTC 6571) and E. coli (NCTC 12241).* The results were determined using broth microdilution after overnight incubation at initial population of $5x10^5$ cfu/ml. The results shown are mean ± standard deviation of three independent replicates.

### 4.2.2 Determination of MIC value for individual phenolic compounds

As shown in **Figure 4-8**, it can be concluded that all of the individual compounds are more active towards *S. aureus* than to *E. coli*. This is consistent with the MIC data of the extracts. Among all compounds tested, EGCg and GA were shown to be highly active in inhibiting the growth of both bacteria, compared to the other two catechin compounds (EC and C). EGCg had an MIC of 62.5 µg/ml and 250 µg/ml when tested on *S. aureus* NCTC 6571 and *E. coli* NCTC 12241 respectively. The results for *S. aureus* are consistent with a previous study which reported MICs between 50 and 100 µg/ml of EGCg when tested on *S. aureus* ATCC 25923 species (Yoda et al., 2004). Both *S. aureus* species NCTC 6571 and ATCC 25923 are in general the same control strains and they have similar MIC values when tested on antibiotics (Andrews, 2001), which then allows a fair comparison with the data from this study. Higher MIC (≥800 µg/ml vs 250µg/ml in this study) was reported for *E. coli* (ATCC 25922) in Yoda et al. (2004) which is probably due

to the different methods used in the MIC assay. Nonetheless, similar results i.e. 250 µg/ml for EGCg MIC was also reported by Nakayama et al.(2011) using *E. coli* NBRC3972 strains.

Of all the banana compounds tested, gallic acid (GA) was found to be the most active towards both bacteria, having MICs of 62.5 µg/ml and 125 µg/ml for *S. aureus* and *E. coli*, respectively. The MIC results for GA on *S. aureus* obtained in this study were consistent with data provided by Gibbons (2004). Catechin and epicatechin, on the other hand, had an equal MIC at 333.33 µg/ml against *S. aureus*. However, higher MIC was recorded on *E. coli* at 1750 µg/ml and 1333.33 µg/ml for catechin and epicatechin, respectively. It is important to note that the MIC of 3-hour extracts towards *E. coli* is similar to the MIC of two major compounds in banana peel extracts. Nevertheless, it is unclear whether the antimicrobial activities exhibited by the extracts on *S. aureus* or *E. coli* were due to individual or combination effects between the compounds in the extracts.

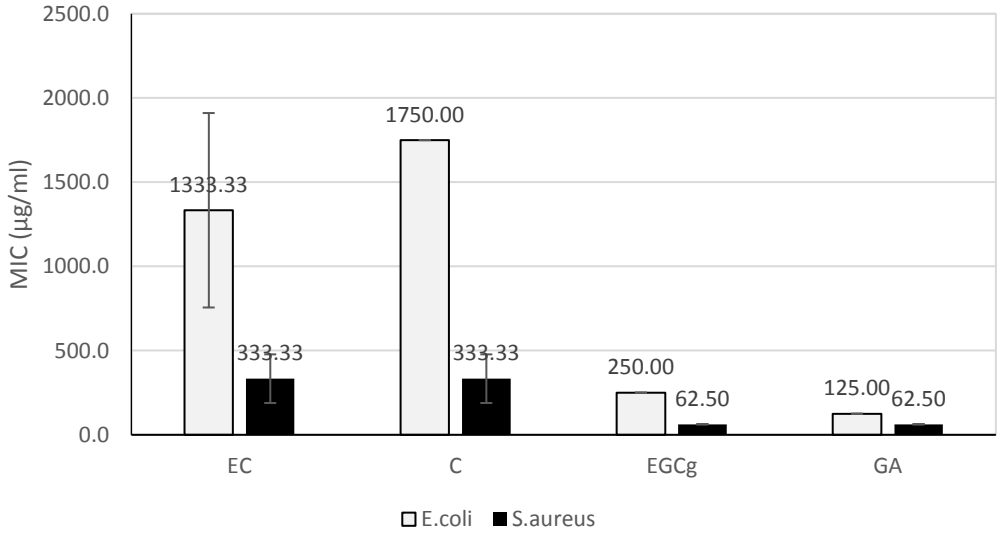



***Figure 4-8:*** *Minimum inhibitory concentration (µg/ml) of individual polyphenols towards E. coli NCTC 12241 and S. aureus NCTC 6571 cells.* The assessment was made using standard of EC: Epicatechin, C: Catechin, EGCg: Epigallocatechin gallate and GA: Gallic acid. The results shown are mean ± standard deviation of three independent replicates.

### 4.2.3 Efficacy of banana peel extracts and individual phenolic compounds in inhibiting *S. aureus* growth

Although some of the MIC data of some of the compounds used in this study have been reported previously, discrepancies between the data are apparent, indicating that MIC assessment on its own cannot be used solely for making decisions about the efficacy of the compounds or the extracts. Such an observation is, however, common in plant antimicrobial studies, because of the diversity in the bacterial strains tested and also the method used for MIC assessment. In order to have a better understanding of the efficacy of these plant-derived compounds as biocides, in this section their performance is evaluated in a series of disinfection treatments towards *S. aureus* bacteria. *S. aureus* in general are round-shaped Gram-positive bacteria, commonly isolated from hospital environments. The selection of *S. aureus* species for the model bacteria in this study was made based on the facts that: (1) it is recognised as the main cause of nosocomial infection in healthcare treatment; (2) it has been ranked as the most prominent cause of many infections in industrialised countries (Kobayashi et al., 2015); (3) recent studies on *S. aureus* species have shown their acquired resistance to commonly used biocides (Seier-Petersen, 2013); and (4) based on data by Public Health England (PHE), *S. aureus* was listed as one of the required bacterial strains used to evaluate the emergence of pathogenic bacteria (Andrews, 2001). Taking this into consideration, *S. aureus* NCTC 6571 strains, as suggested by PHE, were used as one of the test organisms for evaluating the efficacy of the biocides.

In this disinfection study, phosphate buffered saline solution (PBS) was used as the medium for testing the survivability of *S. aureus* after being exposed to increasing doses of extracts and phenolic compounds. Control trials on the survivability of *S. aureus* without the addition of extracts/compounds showed that the PBS used in the disinfection study did not show any inhibition towards *S. aureus* species used in this study (Table C *1*). The pH of the PBS solution was kept constant at 7.2-7.4 for all compounds and extracts tested.

**Figure 4-9** presents the effects of banana peel extracts on the growth of *S. aureus*. A slow reduction in log inactivation of *S. aureus* (< 0.2 log) was noted when the treatment was carried out at its sub-inhibitory concentration (0.5 MIC). Increases in extract doses to 1 MIC and 2 MIC did not statistically improve the inhibitions during the first 2 hours of the treatment ($p<0.05$, when compared to 0.5 MIC). However, more than 0.5 log reduction (70% reduction) was observed after 6 hours' contact time when the extract at 4 MIC was used compared to less than 0.2 log reduction when 0.5 MIC was applied. The finding also indicates that the inhibitory effect of the extracts on *S. aureus* growth followed a dose- and time-dependent manner, and longer time was required for complete inhibition to take place. This is possibly due to the different compounds in the extracts that may perhaps react at different sites of the *S. aureus* cells, before a significant reduction in cell viability could be observed. A similar pattern has been observed in other plant extracts with regard to *S. aureus* growth (Thuille et al., 2003).

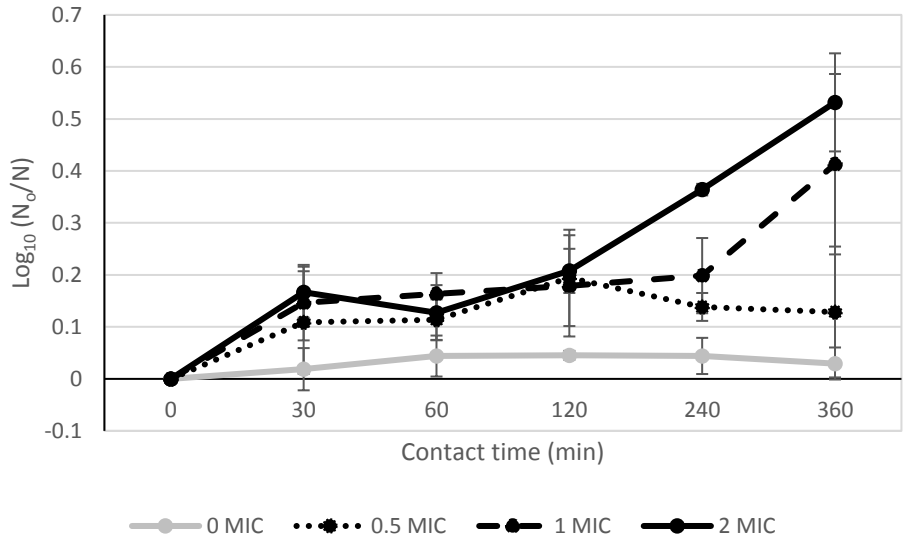



***Figure 4-9:*** *Effect of banana peel extracts (from 3-hour extraction time) on S. aureus growth assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *S. aureus* population after being treated with banana peel extracts. The MIC of banana peel extracts equal to 0.63 mg/ml was determined using broth microdilution. The 0 MIC line represents the untreated control (without the addition of extracts). The error bars represent the standard deviation from three independent experiments.

The results on individual compounds showed that EGCg is the most active in inhibiting *S. aureus* growth, compared to banana compounds (**Figure 4-10**), with increased disinfection time resulting in a higher log reduction. Meanwhile, less pronounced effects were observed in other compounds. It should be noted that EGCg, C and EC are all compounds from flavanol groups, and it has been proposed that the potency of each catechin to bacterial cells could be enhanced by the addition of a gallate moiety to their structure (Tsuchiya, 2001), which possibly accounts for the difference in efficacy between catechins.

To study the effects of EGCg as a biocide, the disinfection treatment was carried out at increasing doses of MIC, and the results are presented in **Figure 4-11**. As observed from the three concentrations tested, EGCg was highly effective in reducing *S. aureus* growth, with increased inactivation ranging from 0.5- to 1.5-log reduction being noted when EGCg was inoculated into *S. aureus* for 30 minutes' contact time, in all concentrations tested. Several studies have proposed different modes of action for EGCg (Zhao et al., 2002; Yoda et al., 2004; Nakayama et al., 2015), and one of them is through binding to peptidoglycan. It is known that *S. aureus* has a thick peptidoglycan membrane, with no outer membrane that blocks its peptidoglycan cell membrane; therefore it is likely that EGCg was able to bind directly to its exposed peptidoglycan cells, reducing the *S. aureus* population immediately after its addition. Moreover, the addition of tea extracts containing 50% EGCg into *S. aureus* was reported to show up-regulation in peptidoglycan proteins (Cho et al., 2008), which then confirmed the interaction of this compound with *S. aureus* cell membranes. The addition of peptidoglycan into the extracellular medium of the *S. aureus* culture has also been found to reduce the bactericidal activity of EGCg, while the addition of lipopolysaccharide (i.e. the outer membrane of *E. coli*) into EGCg had no effect on *S. aureus* growth (Yoda et al., 2004).

This finding also indicates that the antimicrobial activity of EGCg on *S. aureus* follows a dose-dependent relationship, with increased inhibition proportional to the increase in EGCg concentration. For instance, at 2 x MIC of EGCg approximately 1.3-log reduction after 30 minutes was noted, compared to less than 1 log at its MIC concentration. However, increasing

the EGCg dose to 4 x MIC had no significant effects on *S. aureus* inactivation within 30 minutes of incubation ($p>0.05$), indicating that increasing the dose to higher concentrations was not effective for short periods of *S. aureus* and EGCg treatment. Overall, almost 5-log inhibition of *S. aureus* (initial concentration approximately $10^6$ cfu/ml) was achieved after 6 hours' contact time when EGCg at 4 times MIC dose was used. The results also indicate that EGCg was effective in reducing the population of *S. aureus* in the control, with $p<0.05$ for all concentrations tested.

Of all the banana compounds chosen for this study, GA had the lowest MIC value towards *S. aureus* growth compared to catechins. In all concentrations tested (**Figure 4-12**), GA exhibited a non-linear dose-response when the treatment was carried out for longer. For instance, in the log inactivation curve for GA at 2xMIC, an increase in log inactivation was noted immediately after the compound addition, up to 60 minutes, but it reduced as the treatment continued to 2 hours, then it increased again at 4 hours' contact time. The increases and decreases in log inactivation achieved by GA were most likely due to the generation of hydrogen peroxide by the compound. At pH higher than 7, GA undergoes a high degree of oxidation (Pinho et al., 2015), which generates hydrogen peroxide. The addition of hydrogen peroxide to *S. aureus* has been shown to result in an increase in *S. aureus* surface roughness, indicating that hydrogen peroxide interacts with the cell morphology (Cui et al., 2012). Nonetheless, although hydrogen peroxide is known to be a biocide agent, hydrogen peroxide in general has a temporary inhibitory effect on *S. aureus* cells, and at longer treatment times, recovery in cell morphology is evident (Cui et al., 2012). This was seen in this study, as a reduction in log inactivation by GA was noted at 1 hour contact time for 1 MIC and at 2 hour of contact time for 2 MIC and 4 MIC. Overall, less than 0.4-log reduction (60% reduction) was noted in the treatment of GA on *S. aureus*, indicating that GA on its own is a weak inhibitor of *S. aureus*. In a study by Borges et al. (2013), the addition of GA at 100 and 500 µg/ml was shown to result in 23-25% membrane damages to *S. aureus* cells after 30 minutes of treatment. Assuming the same morphology alteration occurred in the *S. aureus* cells, with cell damage being taken as indicative of a reduction in membrane permeability, it is unlikely that ~23% of membrane damage could significantly affect the cells'

viability. This may therefore explain the low inactivation observed in this study, although when the treatment was carried out longer, no higher than 0.4-log reduction (60% reduction) in *S. aureus* was recorded.

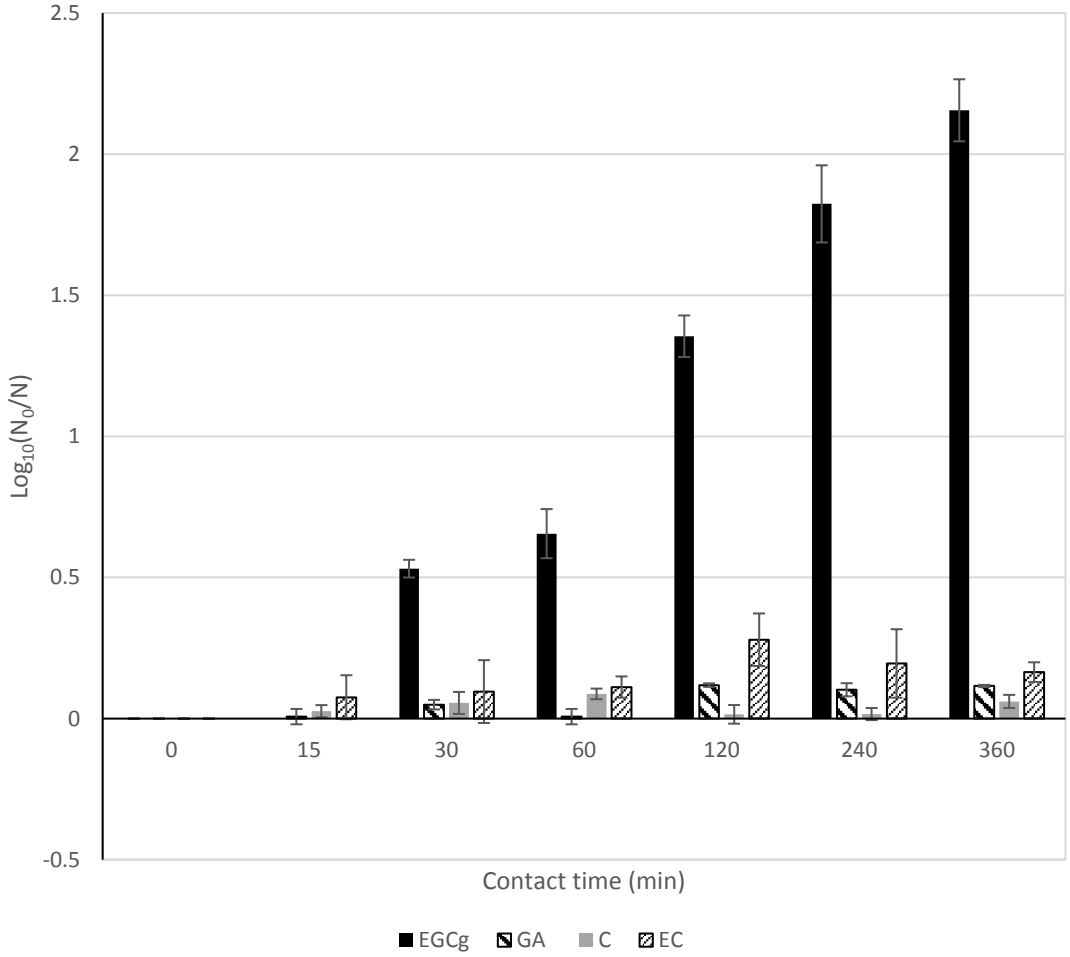



***Figure 4-10:*** *The log reduction of S. aureus treated with individual compounds after 0, 15, 30, 60, 120, 240 and 360 minutes of contact time.* All of the treatment was carried out at compounds' minimum inhibitory concentrations (MICs). The MICs of EGCg, GA, C and EC were 62.5, 62.5, 333.33 and 333.33 µg/ml, respectively, as determined using broth microdilution. Error bars represent the standard deviation of three samples from each disinfection treatment.

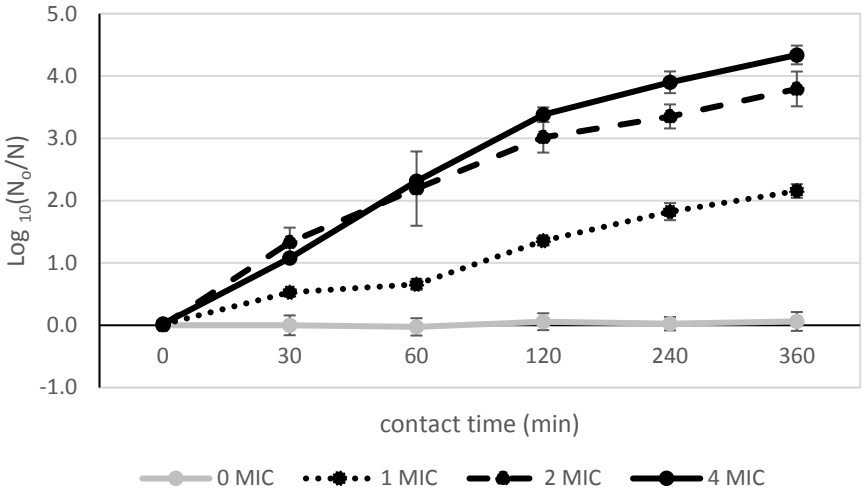



***Figure 4-11:*** *Effect of EGCg on S. aureus growth assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *S. aureus* population after being treated with EGCg. The MIC of EGCg was 62.50 µg/ml, as determined using broth microdilution. The 0 MIC line represents the untreated control (without addition of the compound). All of the EGCg concentration significantly reduced the number of *S. aureus* compared to the untreated control ($p<0.05$). The error bars represent the standard deviation obtained from three independent experiments.

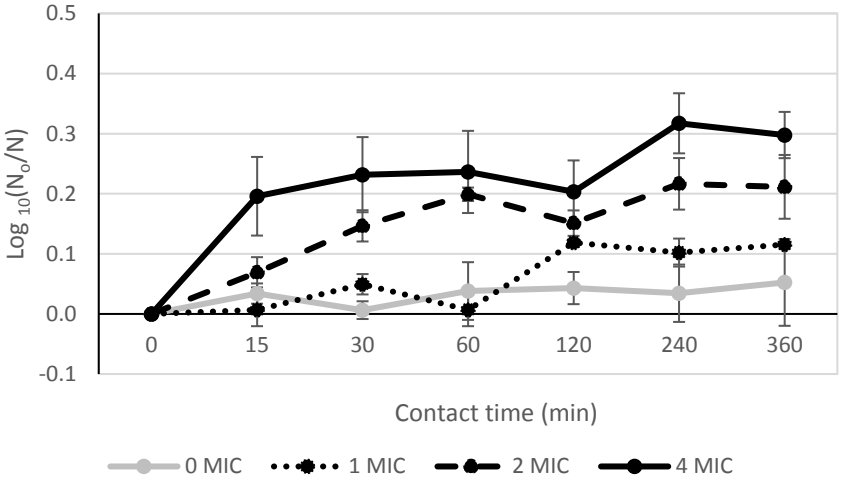



***Figure 4-12:*** *Effect of GA on S. aureus growth assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *S. aureus* population after being treated with GA. The MIC of GA was 62.50 µg/ml, as determined using broth microdilution. 0 MIC represents the untreated control (without addition of the compound). The error bars represent the standard deviation obtained from three independent experiments.

The two catechin compounds (C and EC) tested in this study are isomers, i.e. they have the same molecular formula but different molecular structure configuration. As observed in the in-vitro testing using a 96-wells plate, when both of the compounds were added to $5x10^5$ cfu/ml *S. aureus* population, identical MIC values were recorded. It is known that the biological activities of these catechins are significantly influenced by the difference in their structural configuration (Tsuchiya, 2001). Previous study on catechins' stereospecificity demonstrated that cis-catechins are more effective in reducing membrane fluidity compared to trans-catechins (Tsuchiya, 2001). Indeed the same was observed in this study. As illustrated in **Figure 4-13** and **Figure 4-14**, higher *S. aureus* inactivation (~0.3-log (50% reduction)) was noted at low concentrations (1 x MIC) of EC (i.e. cis-catechin) during 2 hours of contact time, compared to C (i.e. trans-catechin), which was less than 0.10-log (20% reduction). At high doses of EC (i.e 4 MIC), increases in *S. aureus* viability were noted. Although better inhibition was achieved by EC at its MIC, a longer time (~2 hours) is required for the compounds to inhibit *S. aureus* growth. The inhibitory activity of C followed a dose-dependent relationship, with the increase in concentration resulting in higher *S. aureus* inhibition. EC, showed a weaker inhibition than C when a higher concentration was used. A previous study of EC on 18 bacteria species including *S. aureus* NCTC 6571 also showed that at concentrations up to 200 µg/ml, this compound did not show any activity on all of the bacteria tested (Cushnie et al., 2008).

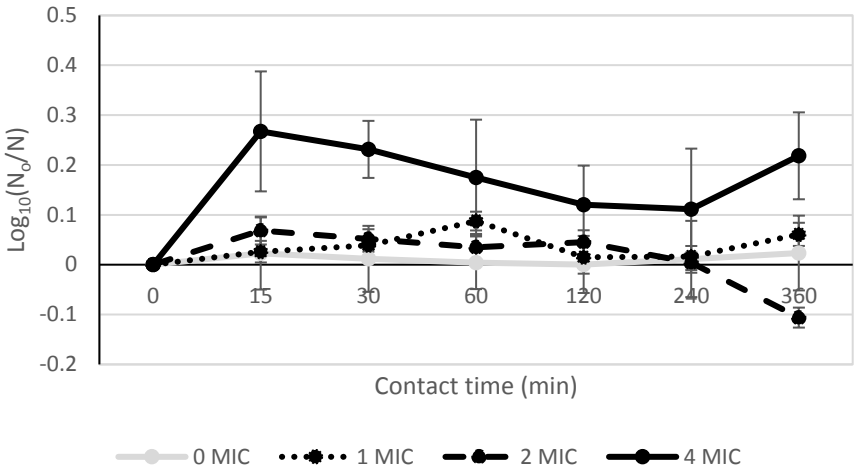



***Figure 4-13:*** *Effect of catechin on S. aureus growth assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *S. aureus* population after treated with C. The MIC of C was 333.33 µg/ml, as determined using broth microdilution. 0 MIC represents the untreated control (without addition of compound). The error bars represent the standard deviation obtained from three independent experiments.

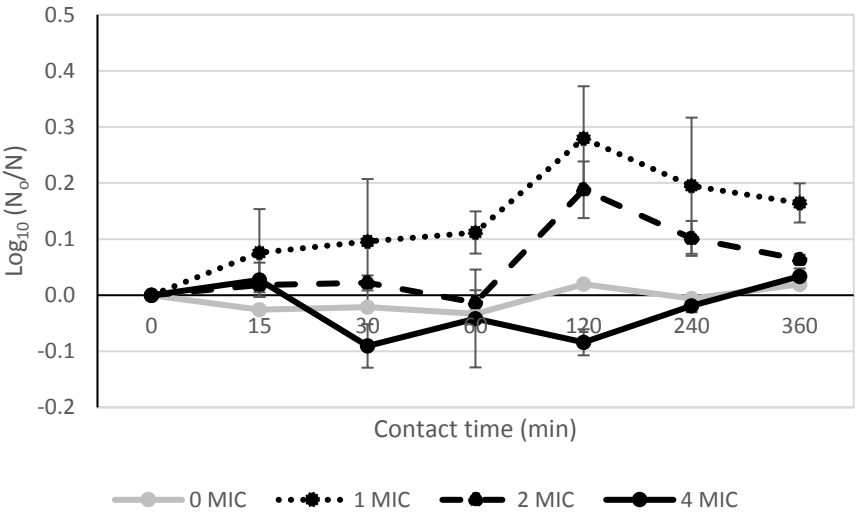



***Figure 4-14:*** *Effect of epicatechin on S. aureus growth assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *S. aureus* population after treated with EC. The MIC of EC was 333.33 µg/ml, as determined using broth microdilution. 0 MIC represents the untreated control (without addition of compound). The error bars represent the standard deviation obtained from three independent experiments.

### 4.2.4 Antimicrobial activity of banana peel extracts and individual compounds on Gram-negative bacteria

Gram-negative bacteria are known in general to be more resistant to plant antimicrobials (Dorman and Deans, 2000). The MIC results on *E. coli* susceptibility in the present study also suggest that a higher dose is required for the antimicrobial activity to take place. It has been proposed by many researchers that the higher tolerance of *E. coli* to many antimicrobial agents, including antibiotics, is due to the additional outer layer in Gram-negative bacteria. This outer membrane, which consists of a lipopolysaccharide (LPS) structure, is known to act as a permeability barrier that prevents many antibiotics from entering the cell membrane. Nevertheless, *E. coli* is recognised as an important bacterial species used to assess the performance of disinfectants, due to the fact that most pathogenic bacteria mostly consist of bacteria from the Gram-negative group (Widsten et al., 2014). Therefore, the antimicrobial activity of the banana peel extracts, together with the individual phenolic compounds, were also be tested on *E. coli* 12241 strains.

Due to the high MIC values (more than 1000 μg/ml) observed in the MIC data on two major banana extract-derived compounds, C and EC, and also their weak inhibitory activity towards *S. aureus*, these two compounds were excluded from the *E. coli* disinfection study. Nonetheless, in order to allow a better assessment of the inhibitory effects of two selected phenolic compounds against *E. coli* cells, a comparative study on EGCg and GA was carried out towards two other Gram-negative bacteria, *A. hydrophila* and *B. cepacia*. *A. hydrophila* and *B. cepacia* were isolated from Hyde Park pond water. In general, these two natural bacteria are commonly found in water, with *B. cepacia* known to be an opportunist pathogen. All experiments involving *A. hydrophila* and *B. cepacia* identification and MIC determination were carried out by Huiyun Niu, an MSc student co-supervised by the author and summarised further in the MSc dissertation thesis entitled *Natural biocides derived from banana peel extracts: effects on heterotrophic plate count (HPC)*.

#### *4.2.4.1 Effectiveness of banana extracts in inhibiting E. coli growth*

Banana peel extracts had an MIC value of 1.25 mg/ml when tested on *E. coli*. Under the same disinfection conditions as tested against *S. aureus* (**Figure 4-15**), the addition of banana extracts at their MIC concentration showed a limited antimicrobial activity on the *E. coli* growth when assessed with 6 hour contact time. Although a reduction in *E. coli* growth up to 0.1-log reduction (20% reduction) was observed at the banana extracts' MIC during the first hour of the treatment, an increase in *E. coli* growth was noted when the treatment was extended ($p<0.05$). This indicates that with a longer treatment time, some of the compounds in the extracts may favour *E. coli* growth (e.g. as nutrients to support bacterial growth). Increasing the extract dose to 2xMIC managed to overcome the regrowth effect, and yet weak inhibition was noted during the first two hours of the treatment (<0.1-log reduction (20% reduction)). Nevertheless, increasing the banana peel extract dose to 4 x MIC significantly improved the disinfection performance ($p<0.05$), and resulted in higher *E. coli* inhibition (~0.3-log reduction was observed (50% reduction)).

The results indicate that the activity of banana peel extracts on *E. coli* growth follows a dose-dependent manner, though extremely high concentrations of banana peel extracts are required for complete inhibition of *E. coli* growth. As can be seen, at the maximum concentration of 5 mg/ml for the banana extracts tested in this study, only up to 0.3-log reduction (50% reduction) in *E. coli* growth was noted after 6 hours of disinfection treatment. This may be because the banana extracts mainly consist of polar compounds (Kanazawa and Sakakibara, 2000; Someya et al., 2002; Mokbel and Hashinaga, 2005), which are known to have limited affinity to bacterial membranes, particularly those of Gram-negative bacteria. This is possible as higher inactivation of *E. coli* (> 2-log reduction) was noted when non-polar extracts (Neem oil) were tested at a dose of 2.13 mg/ml with just 5 minutes of contact time (Matthews et al., 2009). Non-polar antimicrobials, either in a mixture or as individual compounds, are known to have higher affinity to the lipid structure of bacterial cells, compared to polar compounds (Negi, 2012). Some researchers also suggest that the low inhibitory effect exhibited by phenolic compounds is

due to the low uptake of the compounds at pHs above 7. Nonetheless, in a study by Sanchez-maldonado (2014), the author found that even in the absence of phenolic compounds, no growth in *E. coli* was observed at a pH of 5, suggesting that *E. coli* cells are compromised at pH values lower than 5.

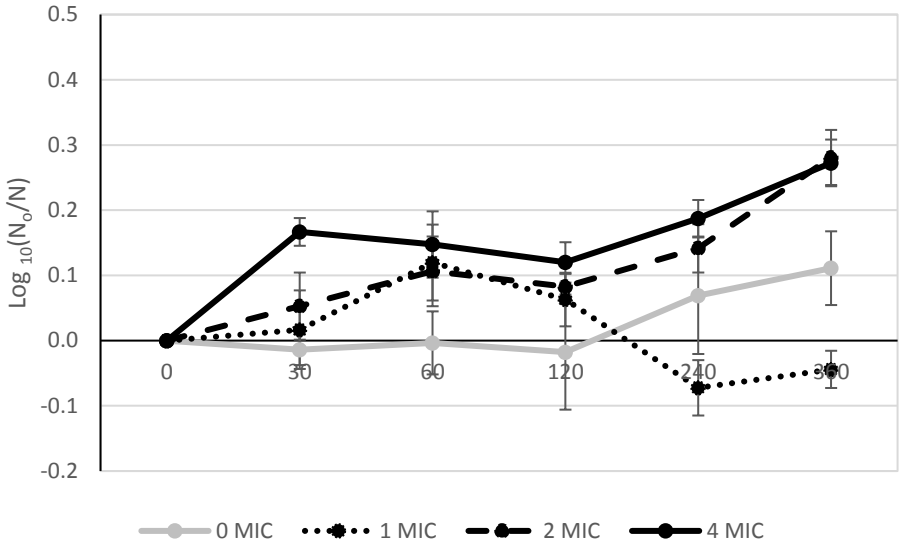



***Figure 4-15:*** *Effect of banana peel extracts on E. coli growth, assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *E.coli* population after treated with banana extract. The MIC of banana peels extract was 1.25 mg/ml, as determined using broth microdilution. 0xMIC represents the untreated control (without addition of extracts). The error bars represent the standard deviation obtained from three independent experiments.

#### *4.2.4.2 Effectiveness of epigallocatechin gallate (EGCg) in inhibiting Gram-negative bacteria*

The effects of EGCg on *E. coli* growth are less pronounced than those on *S. aureus*, with slow inhibitory activity (less than 0.2 logs (40% reduction)) being observed at its MIC during the early stage of the treatment (up to 4 hours). It then started to increase dramatically beyond that contact time (**Figure 4-16**). The same trends were followed in all concentrations tested, indicating that the interaction between EGCg and *E. coli* was time-dependent rather than dose-dependent. The highest *E. coli* inhibition (more than 1 logs) was noted after 6 hours of disinfection treatment at 4 x MIC (~1000 µg/ml). The effects of EGCg on *E. coli* cell morphology have been studied previously by Nakayama et al. (2013). The authors proposed that EGCg interacts with LPS cells by blocking the porins sites known as OmpG, which are responsible for the transfer of small hydrophilic molecules required for the cells' metabolism process. Therefore, it is likely that low log reduction, as observed during the first two hours, may be due to the binding process between EGCg and the target sites (porins), before a significant reduction in cell viability can be observed. Indeed, in the same study, the authors also demonstrated that at a concentration of 1000µg/ml EGCg, at least one hour's contact time is required before a significant reduction in cell metabolism was evident (this was monitored through the amount of glucose uptake in the cells) (Nakayama et al., 2013). Nevertheless, the *E. coli* MIC result obtained from the present study was consistent with their study at 250 µg/ml, even though different *E. coli* strains were used, which implies that this binding phenomenon may also occur in the *E. coli* strains used in the present study.

The *E. coli* strains used in this present study were also found to have higher resistivity to EGCg compared to other Gram-negative bacteria. EGCg was shown to inhibit *S. maltophilia* growth at a similar MIC to *E. coli* used in this study, and yet up to 1-log *S. maltophilia* reduction was achieved during 5-hour treatment at 256 µg/ml EGCg dose (Gordon and Wareham, 2010). Although the precise mechanism of EGCg antimicrobial activity on Gram-negative bacteria still remains unclear, one of the proposed mechanism of EGCg to *E. coli* has been reported to be due

to the generation of hydrogen peroxide (Cui et al., 2012). Since both *E. coli* and *S. maltophilia* are catalase-positive Gram-negative bacteria, they are able to inhibit hydrogen peroxide stress produced by EGCg by producing catalase and converting the hydrogen peroxide to water and oxygen (Imlay, 2008). However, *E. coli* has a unique tolerance to hydrogen peroxide exposure. It has been reported that interaction of *E. coli* with hydrogen peroxide, even at low concentrations, is able to activate a defence protein, superoxide dismutase (SOD), in cells (Imlay, 2008). Therefore it is likely that this defence mechanism was responsible for the resistance of *E. coli* to EGCg. This may also be the factor that prevented EGCg from effectively reducing *E. coli* growth compared to *S. maltophilia*. Indeed, regarding the effects of EGCg on *E. coli* cell morphology, full recovery in the cell morphology also was noted after two hours of treatment (Cui et al., 2012), indicating that an increase in the oxidative stress response allows *E. coli* strains to overcome the inhibitory effect of EGCg.

In order to have a better understanding of the phenomenon, and to determine whether it is only specific to *E. coli* species, a similar experimental set-up was carried out using two Gram-negative bacteria species: *A. hydrophila* and *B. cepacia*. The results for EGCg effects on these two bacteria species are presented in **Figure 4-17** and **Figure 4-18**. The MICs of EGCg on *A. hydrophila* and *B. cepacia* were recorded as 15.6 and 62.5 µg/ml, respectively. Between these two bacteria species, *A. hydrophila* was found to be more susceptible to EGCg treatment, with MIC values lower than the lab-strain *E. coli*. Nevertheless, weaker inhibitions (< 0.5-log reduction (70% reduction)) were obtained when *A. hydrophila* were tested with the same experimental procedure as in *E. coli*. Also, higher *A. hydrophila* inhibition could only be seen when the treatment was extended up to 6 hours. The same trends were also observed in *E. coli*. *B. cepacia* is an opportunistic pathogen in water, therefore the effect of EGCg on *B. cepacia* is expected to be lower than that on *A. hydrophila* and *E. coli*. As illustrated in **Figure 4-18**, *B. cepacia* are more resistant to EGCg as less than 0.1-log reduction (20% reduction) was achieved in all concentrations tested.

It is generally observed that most natural bacteria isolates are more resistant to biocides than lab-strain bacteria species (Mushantaf et al., 2012). This therefore may also explain why weak inhibitory activity was exerted by EGCg on *A. hydrophila* and *B. cepacia*, compared to *E. coli* in this study. Taking all these results together, this finding also indicates that disinfection treatment using EGCg is generally ineffective towards Gram-negative bacteria, and the effects are not only specific to *E. coli*, although higher inactivation was achieved in lab-strain bacterial species. In addition, it can be concluded that a longer contact time and higher concentrations of EGCg are required to fully inhibit Gram-negative bacteria growth. The resistance of Gram-negative bacteria to EGCg has been hypothesised to be due to their negatively charged surface membrane, which lowers the ability of EGCg to bind to the cells (Ikigai et al., 1993). It is worth noting that (at the pHs used in this study), EGCg was also in a negatively charged state.

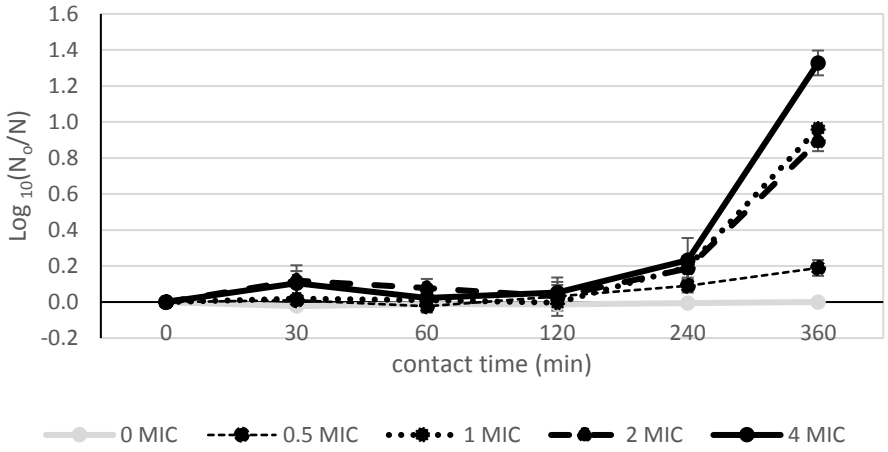



***Figure 4-16:*** *Effect of EGCg on E. coli growth, assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *E.coli* population after treated with EGCg. The MIC of EGCg to *E.coli* was 250 µg/ml, as determined using broth microdilution. 0xMIC represents the untreated control (without addition of compound). None of the treatments statistically reduced the population of *E.coli* ($p>0.05$) when compared to untreated control. The error bars represent the standard deviation obtained from three independent experiments.

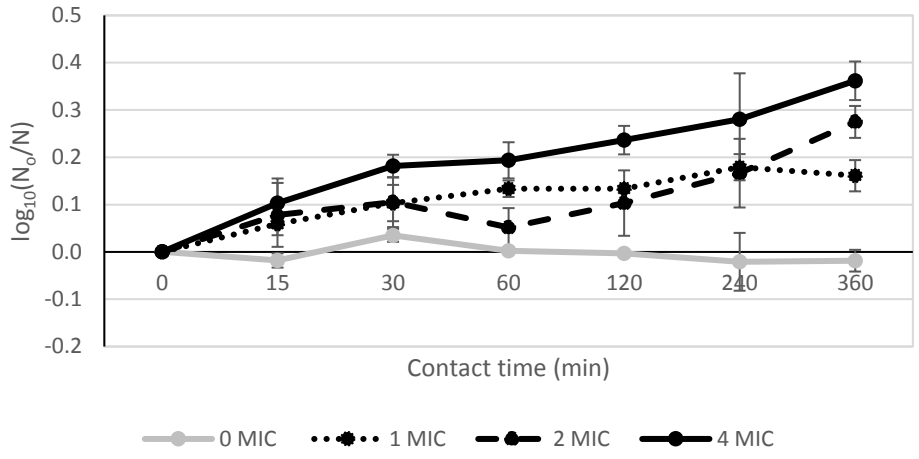



***Figure 4-17:*** *Effect of EGCg on A. hydrophila growth, assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *A. hydrophila* population after treated with EGCg. The MIC of EGCg to *A. hydrophila* was 15.6 µg/ml, as determined using broth microdilution. 0 MIC represents the untreated control (without addition of compound). The error bars represent the standard deviation obtained from two independent experiments.

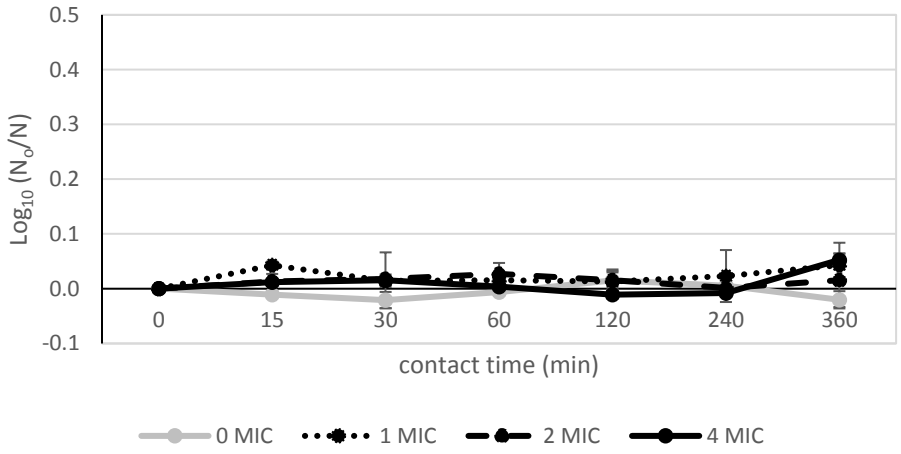



***Figure 4-18:*** *Effect of EGCg on B. cepacia growth, assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *B. cepacia* population after treated with EGCg. The MIC of EGCg to *B. cepacia* was 62.5 µg/ml, as determined using broth microdilution. 0 MIC represents the untreated control (without addition of compound). The error bars represent the standard deviation obtained from two independent experiments.

#### *4.2.4.3 Effectiveness of gallic acid (GA) in inhibiting Gram-negative bacteria*

As illustrated in **Figure 4-19**, the antimicrobial activity of GA on *E. coli* species can be considered as weak with less than 0.1 logs reduction (20% reduction) being observed in all concentrations tested. At a higher concentration of GA (i.e. 2 x MIC), increased *E. coli* growth was noted, suggesting that the 're-growth effects' as observed in the extracts may partly be due to the interaction of GA with *E. coli* cells (i.e. the GA may be supporting growth as a substrate). Increases in the GA dose made no significant difference to the log inactivation ($p>0.05$), suggesting that regardless of the dose, GA is not an effective inhibitor for *E. coli*. Using different *E. coli* strains, Wang et al. (2017) also observed the weak inhibitory activity of GA at a concentration of 15 mM. The authors suggested that the low inhibitory effect exhibited by GA was likely to be due to the low uptake of GA by the bacteria at neutral pH (pH~7.4). The same pH was also used in the present study.

Phenolic acids in general can cross bacterial cell membranes through the passive diffusion of their undissociated form (Campos et al., 2009; Wang et al., 2017). At a neutral pH, however, the carboxyl group ($pK_a=4.0$) was dissociated, which subsequently reduces the ability of the compounds to cross the membrane and limits their uptake in the bacteria cell membranes. This phenomenon, however, is inconsistent with a previous study that showed higher inactivation on Gram-negative bacteria when the cells were tested at pH 8 compared to pH 3, 5 and 7, with the lowest activity being observed at pH 3 (Pinho et al., 2015). Based on this finding, it is therefore unclear whether the low activity exhibited by the GA was pH-dependent, or whether it was due to the defence mechanism of *E. coli* when exposed to hydrogen-peroxide-producing inhibitors such as GA. Indeed, *E. coli* JM109, which is a recA deficient strain, showed higher inactivation ~up to 3 logs reduction when tested with GA at 4.5 mg/ml (Diaz-Gomez et al., 2014). recA is a protein responsible for DNA repair and maintenance in cells. *E. coli* deficient in recA are known to be highly sensitive to hydrogen peroxide (Asad et al., 2004).

In order to have a better understanding of GA inhibitory activity, similar disinfection was carried out using other Gram-negative bacteria as shown in **Figure 4-20** for *A. hydrophila*, and

**Figure 4-21** for *B. cepacia*. The MICs of GA on *A. hydrophila* and *B. cepacia* were recorded at 7.8 and 31.3 µg/ml, respectively. As observed, higher inhibitions were exhibited by the *A. hydrophila* (more than 0.20-log reduction (~40% reduction)), compared to *E. coli* (maximum inhibitions at ~0.10-log (20% reduction)), indicating that GA was able to reduce Gram-negative growth, though only weakly. The low inhibitions of *E. coli* may be due to the defence mechanism against hydrogen peroxide. Similar to EGCg, GA also has limited activity for reducing the growth of the opportunist pathogen *B. cepacia*.

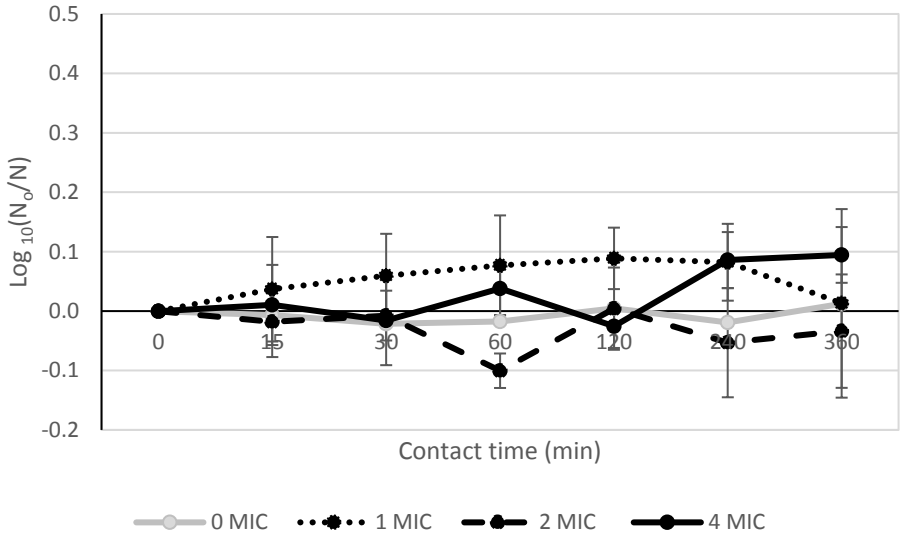



***Figure 4-19:*** *Effect of GA on E. coli growth, assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *E.coli* population after treated with GA. The MIC of GA to *E.coli* was 125.0 µg/ml, as determined using broth microdilution. 0 MIC represents the untreated control (without addition of compound). Only at 1 MIC of GA, the number of *E. coli* population was significantly reduced, when compared to the untreated control ($p<0.05$). The error bars represent the standard deviation obtained from three independent samples.

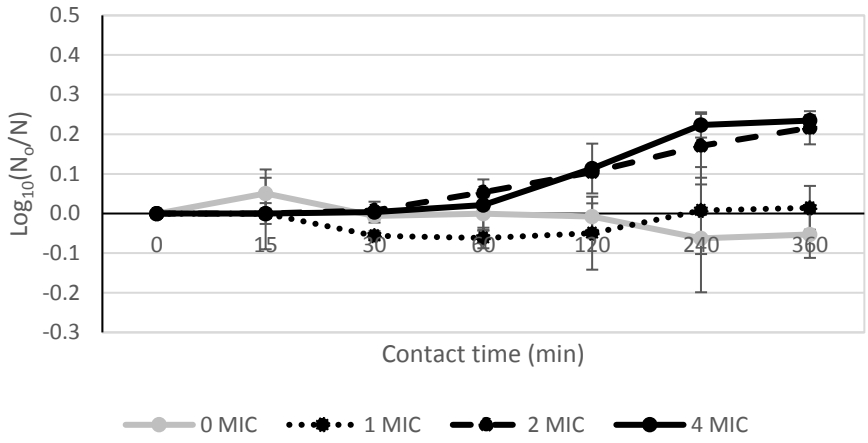



***Figure 4-20:*** *Effect of GA on A. hydrophila growth, assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *A. hydrophila* population after treated with GA. The MIC of GA to *A. hydrophila* was 7.8 µg/ml, as determined using broth microdilution. 0 MIC represents the untreated control (without addition of compound). The error bars represent the standard deviation obtained from two independent experiments.

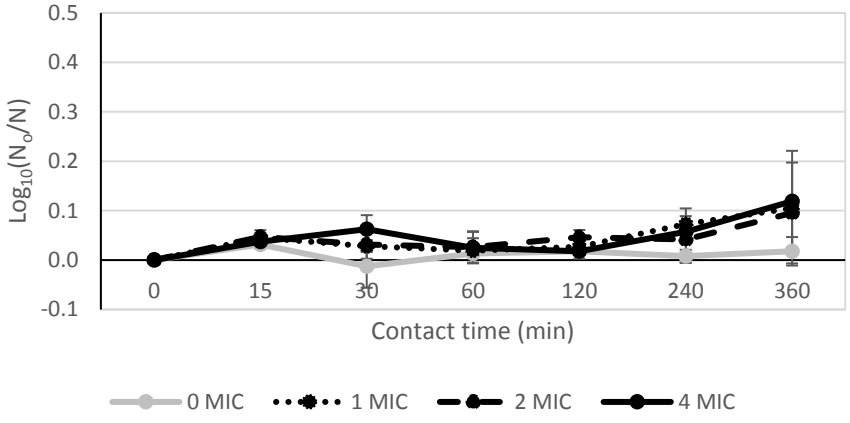



***Figure 4-21:*** *Effect of GA on B. cepacia growth, assessed in 6-hr disinfection treatment.* The y-axis represents the log reduction of *B. cepacia* population after treated with GA. The MIC of GA to *B. cepacia* was 31.3 µg/ml, as determined using broth microdilution. 0 MIC represents the untreated control (without addition of compound). The error bars represent the standard deviation obtained from two independent experiments.

## 4.3 Synergies between banana extract-derived antimicrobials

As shown in the previous chapter, most of the banana extract-derived compounds exhibit only a low inhibitory activity on bacteria growth. Although some do show promising activity (particularly GA and EC against *S. aureus*), the time taken to achieve satisfactory reductions in bacteria counts are too long to be practically useful. Some researchers believe that antimicrobial compounds in plants do not work on their own, but rather through the combination of their synergistic activity (Lewis and Ausubel, 2006; Vuuren et al., 2011). This is supported by findings on EOs, which showed higher inhibitory activity on Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) when a mixture of EOs is used instead of their individual extracts (Delaquis et al., 2002). Moreover, in a review study by Vuuren et al. (2011), the authors also concluded that in most cases, the activity of crude extracts was found to be higher than that of their isolated compounds. Inhibitory activity between the banana extracts and their individual compounds, observed through the MIC study, also indicated that the inhibitory activity of the crude extracts was better than the major individual compounds alone (i.e. EC and C) on *E. coli*.

To gain a better understanding of the interaction between the identified compounds, as well as their activity, a combination study on the compounds against both of the studied bacterial species (*S. aureus* NCTC6571 and *E. coli* NCTC12241) was carried out. Since catechin (C) and epicatechin (EC) are from the same flavanol group, their combination activity was not tested together as they may have similar target sites on bacteria membranes (Tsuchiya, 2001), but they were tested with GA instead. Similar to the disinfection study which used individual compounds, for the effects of compounds in combination, the effects of GA in combination with EGCg will be presented in this section. The final combination study was as follows: EC-GA, C-GA and EGCg- GA. The selection of these combinations was made based on consideration of individual compounds that may have different antimicrobial mechanism or target sites, and based on the inhibitory activity exerted by each of the compounds tested when they act alone, as shown in sections **4.2.3** and **4.2.4**.

Owing to the complexity of the combination study, in this present study the interactions between the individual compounds were tested using a similar MIC set-up as the one in the individual test, with the exception that two-fold dilutions of the compounds were added manually into each well in a decreasing order, thereby making each well a unique combination of two-fold addition. The MICs of the compounds' combinations were compared with the response of each individual compound alone, and ∑FIC was interpreted according to Vuuren et al.,'s (2011) definition. It should be noted that the combination activity in this study was limited to two inhibitors. Some of the combinations with synergy interaction were then tested following the disinfection protocol described in **3.2.7**, in order to evaluate their performance as a biocide in six-hour disinfection treatment.

Using isobolograms methods, the synergy interaction and the combined activity are presented in **Figure 4-22** and **Figure 4-23**. As described in 3.3.2, synergy was considered for data points that fell below the 0.5:0.5 line. As can be seen, some synergy activities were noted in combinations of the compounds with *S. aureus* species (**Figure 4-22**), but none of the combinations produced any synergy with *E. coli* (**Figure 4-23**). This may be due to the low inhibitory activity exerted by the compounds when they were tested individually, and also the high tolerance of Gram-negative bacteria to most antimicrobial compounds. Consistent with the observed results, a combined treatment of a sub-inhibitory dose of GA with C at its inhibitory concentrations also showed similar growth inhibition halos with catechin alone, indicating that the addition of GA to C had no synergy in *E. coli* inactivation (Diaz-Gomez et al., 2014). The synergy interaction observed in the *S. aureus* treatment was then tested in detail with a 6 hours' disinfection treatment, and the results are presented in **Figure 4-24**, **Figure 4-25** and **Figure 4-26**. The raw data for compound combinations to *E. coli* is provided in **Appendix D1: Raw data for compounds combination to *E. coli***.

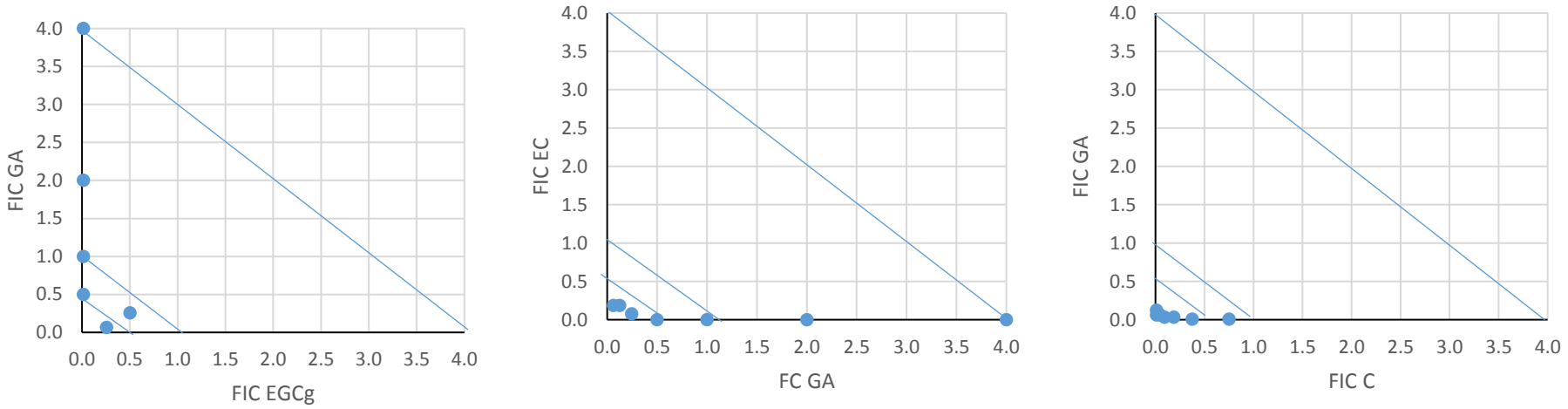



***Figure 4-22**: Isobologram of (left) GA-EGCG (middle) EC-GA (right) GA-C, to S. aureus growth.* The combination of compounds was considered to have achieved synergy if the data points fell below the 0.5:0.5 line.

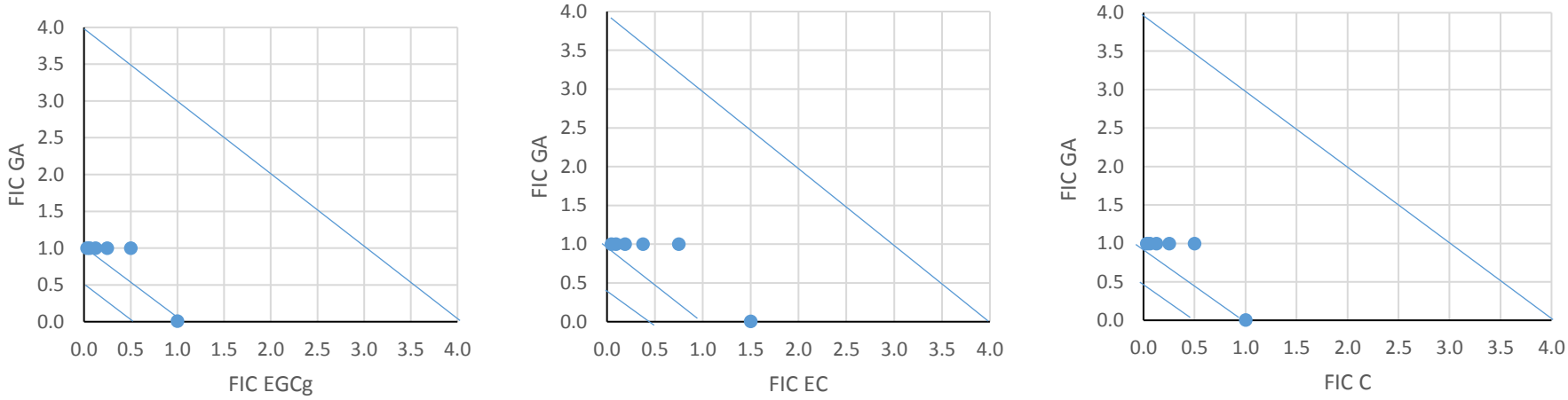



***Figure 4-23:*** *Isobologram of (left) GA-EGCg,(middle) GA –EC (right) GA-C, to E. coli growth.* The combination of compounds was considered to have achieved synergy if the data points fell below the 0.5:0.5 line.

### 4.3.1 Combination of epigallocatechin gallate (EGCg) and gallic acid (GA) against *S. aureus* growth

Taking ∑FIC ≤0.5 to depict the synergy between antimicrobials, only one combination of EGCg and GA resulted in a synergy interaction (combination 1), while the combination of 31.3 µg/ml EGCg-16 µg/ml GA (combination 2) and 1 µg/ml EGCg-31.3 µg/ml GA (combination 3) is additive ($0.5 \leq \sum FIC \leq 1.0$) as shown in **Table 4-2**. From the ∑FIC data, these combinations were then tested for their disinfection efficiency on *S. aureus* and the results are presented in **Figure 4-24**. It is interesting that even though in combination 2:31.3 µg/ml EGCg + 16 µg/ml GA was interpreted as additive instead of synergetic, an improved inhibitory activity of EGCg-GA was noted when it was combined compared to the activity of the compounds alone ($p<0.05$). This finding is consistent with the earlier definition of synergy between antimicrobial agents by Berenbaum (1978), where he stated that synergistic interaction between agents can be considered when the ∑FIC is less than 1.

In general, most of the EGCg and GA selected combinations showed enhanced inhibitory activity towards *S. aureus* with more than 1-log reduction being observed within 15 minutes in all EGCg-GA combinations except in combination 3. As shown in **Figure 4-24**, most combinations of EGCg-GA effectively reduced the *S. aureus* population ($p<0.05$) compared to the control. Both compounds in this combination study had an equal MIC of 62.5 µg/ml when tested alone on *S. aureus*; however, limited inhibition was observed for GA.

Combined treatment of EGCg and GA at 16µg/ml and 4µg/ml (respectively) showed a synergy interaction with ∑FIC of 0.32, with more than 5-log reduction after 2 hours compared to 2-log in the EGCg individual treatment. Using the same ratio of EGCg and GA, the treatment was repeated by doubling up the dose of EGCg and GA as illustrated in combination 4. As expected, the increase in compound dose led to an increase in disinfection log reduction with higher inhibition (>2 log reduction) during 15 and 30 minutes although as the treatment was continued for longer, a reduction in its efficacy was evident. This may be due to the instability of GA activity at longer treatment time, as observed in its individual treatment.

Unlike EGCg, GA is a weak *S. aureus* inhibitor. The present study also showed that low inhibitions in *S. aureus* growth are achieved when GA is used alone. Despite its weaknesses as an *S. aureus* inhibitor, GA is a membrane-active compound (Borges et al., 2013). Although the exact mechanism is still unknown, it is believed that GA can cause cells lyses through the binding and disruption of cell integrity (Monte et al., 2014). Moreover, the addition of GA to the *S. aureus* culture has been shown to increase cells' hydrophobic character (Borges et al., 2013). Therefore it is likely that the cell hydrophobicity induced by GA allows less polar compounds such as EGCg to enter the cells easily.

Indeed, the combined treatment of EGCg-GA at a higher GA dose (31.3 µg/ml) as shown in combination 3 had no improved activity to *S. aureus* log inactivation, indicating that the GA in GA-EGCg was not solely responsible for the growth inhibition of the *S. aureus*. This observation therefore suggests that the improvement in EGCg reactions to *S. aureus* could be due to the presence of traces of GA in the solution, which induces its entry into cells. To the author's knowledge, no studies have been carried out on a combination of GA with any other plant-derived compounds against *S. aureus* species that could allow justification of the observation made in this study. Nevertheless, Lee and Je (2013) also observed that the addition of GA to chitosan showed a remarkable improvement in *S. aureus* inhibition compared to the activity of chitosan alone. Similar to this present study, their study also demonstrated that increasing the dose of GA (from 53.87 to 118.92 µg/ml) made no difference to the activity, suggesting that GA is more effective at low concentrations for synergetic activity to occur.

***Table 4-2**: Fractional inhibitory concentration (∑FIC) of EGCg combined with GA*

| MIC EGCg in combination (µg/ml) | MIC GA in combination (µg/ml) | FIC EGCg | FIC GA | ∑FIC | |
|---|---|---|---|---|---|
| 1 | 250 | 0.016 | 4 | 4 | |
| 1 | 125 | 0.016 | 2 | 2 | |
| 1 | 62.5 | 0.016 | 1 | 1 | |
| 1 | 31.3 | 0.016 | 0.5008 | 0.5168 | Combination 3 |
| 31.3 | 16 | 0.5008 | 0.256 | 0.7568 | Combination 2 |
| 16 | 4 | 0.256 | 0.064 | 0.32 | Combination 1 |

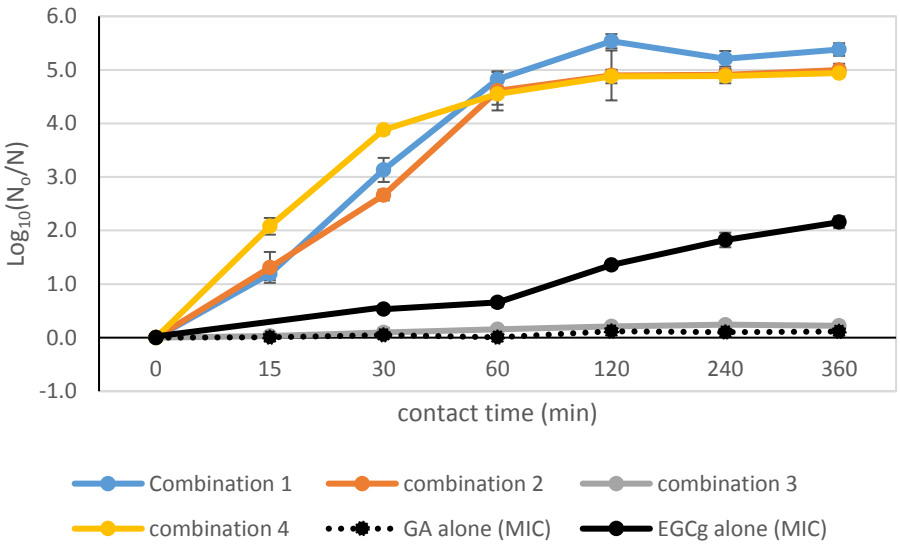



***Figure 4-24:** Combination of EGCg and GA to S. aureus. Combination 1: 16µg/ml EGCg +4 µg/ml GA; Combination 2: 31.3 µg/ml EGCg + 16 µg/ml GA; Combination 3: 1 µg/ml EGCg + 31.3 µg/mlGA; Combination 4: 32 µg/mlEGCg + 8µg/ml GA.* The y-axis represents the log reduction of *S. aureus* due to the treatment. The error bars represent the standard deviation obtained from three independent experiments.

### 4.3.2 Combination of epicatechin (EC) and gallic acid (GA) against *S. aureus* growth

Out of seven combinations of GA-EC tested in this study (**Table 4-3**), only three were found to have $\sum FIC \leq 0.5$; i.e. synergy interaction. From **Table 4-3**, additive and indifferent effects were observed when GA was added at concentrations beyond 31.25 µg/ml (¼ of its MIC). EC had an MIC of 333.33 µg/ml when tested alone on *S. aureus*. Combination of subinhibitory concentration of EC as showed in combination 1 and 3, made no difference to the *S. aureus* growth, with weaker inhibitory activity with less than 0.2-log reduction being noted, although when the treatment was extended to six hours (**Figure 4-25**). However, when the disinfection was continued at a higher GA concentration at 8 µg/ml (combination 2), a slight improvement in log reduction was observed with almost 0.4-log reduction (60% reduction) being noted at the end of the disinfection, suggesting that GA may play an important role in the interaction between EC and *S. aureus*. Nevertheless, the combined treatment of GA and EC as observed in combination 2, did not statistically differ from EC individual treatment ($p>0.05$). It should be noted that, the log inhibitory pattern exhibited by the combination of GA and EC are consistent with those observed in the extracts (i.e. increases in contact time, resulted in higher log reduction), suggesting that the activity observed in the extracts may be due to the interaction between EC and GA. Using membrane system liposomes, Nakayama et al. (1998) demonstrated that GA and EC on their own were unable to penetrate cell membranes, which may explain the poor activity observed for the compounds individually. As observed in the performance of EC or GA (**Figure 4-14** and **Figure 4-12**, respectively) when they were used alone, where fluctuations in their inhibitory activity were noted, the combination of these two compounds managed to sustain the antimicrobial activity, which may suggest that these compounds may work in synergy in inhibiting the growth of *S. aureus*.

Overall, the results indicate that EC can be effectively used at much lower concentrations than its MIC when used together with GA, and it is likely that the addition of GA causes more effective binding between EC to *S. aureus* cells, due to GA's ability to interfere with cell hydrophobicity, although at high GA concentrations (i.e. in combination 3) the opposite effects were observed.

***Table 4-3**: Fractional inhibitory index (∑FIC) of GA combined with EC*

| MIC GA in combination (µg/ml) | MIC EC in combination (µg/ml) | FIC GA | FIC EC | ∑FIC | |
|---|---|---|---|---|---|
| 250 | 0.5 | 4 | 0.0015 | 4.0015 | |
| 125 | 0.5 | 2 | 0.0015 | 2.0015 | |
| 62.5 | 0.5 | 1 | 0.0015 | 1.0015 | |
| 31.25 | 8.0 | 0.5 | 0.0240 | 0.5240 | |
| 15.3 | 26.2 | 0.2448 | 0.0786 | 0.3234 | Combination 3 |
| 7.81 | 62.5 | 0.12496 | 0.1875 | 0.3125 | Combination 2 |
| 4 | 62.5 | 0.064 | 0.1875 | 0.2515 | Combination 1 |

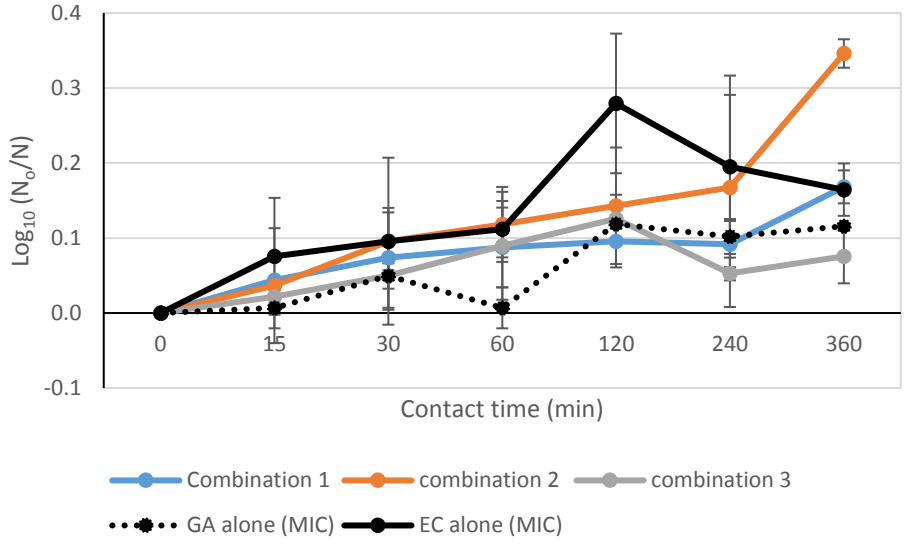



***Figure 4-25:** Combination of GA and EC on S. aureus growth. Combination 1: 4 µg/ml GA-62.5 µg/ml EC; Combination 2: 8 µg/ml GA-62.5 µg/ml EC; Combination 3: 15µg/ml GA-26µg/ml EC.* The y-axis represents the log reduction of *S. aureus* population due to the treatment. The error bars represent the standard deviation obtained from three independent experiments.

### 4.3.3 Combination of catechin (C) and gallic acid (GA) against *S. aureus* growth

As shown in **Table 4-4**, only one combination of C and GA did not have synergy, while the rest of the combinations were synergetic, agreeing with Vuuren et al. (2011). However, in order to allow better graphical interpretation, only three of the combinations with least amount of GA and C were tested in the disinfection study towards *S. aureus* growth (**Figure 4-26**). When compared to the activity of the compounds alone, from three combinations tested, two of them (combination 2 and combination 3) showed a higher log reductions compared to the performance of C and GA alone. For instance, combination of equal ratios of C and GA (combination 2), resulted in up to 0.3-log reduction (50% reduction) at the end of the treatment compared to less than 0.2-log (40% reduction) in the treatment of individual compounds ($p<0.05$).

Although slightly lower reductions were noted in the other two combinations (i.e. combination 1 and combination 3), the results confirm that the selected banana peel-derived compounds used in this study work in synergy. This also consistent with higher inhibitory observed in the extracts towards *S. aureus* compared to the compounds alone.

***Table 4-4**: Fractional inhibitory concentration (∑FIC) combination of GA with C*

| MIC C in combination (µg/ml) | MIC GA in combination (µg/ml) | FIC C | FIC GA | ∑FIC | |
|---|---|---|---|---|---|
| 250 | 0.5 | 0.75 | 0.01 | 0.76 | |
| 125 | 2.0 | 0.38 | 0.03 | 0.41 | |
| 62.5 | 4.0 | 0.19 | 0.06 | 0.25 | |
| 31.25 | 4.0 | 0.09 | 0.06 | 0.15 | |
| 15.63 | 4.0 | 0.05 | 0.06 | 0.11 | Combination 1 |
| 7.81 | 8.0 | 0.02 | 0.13 | 0.15 | Combination 2 |
| 3.91 | 16.0 | 0.01 | 0.26 | 0.27 | Combination 3 |

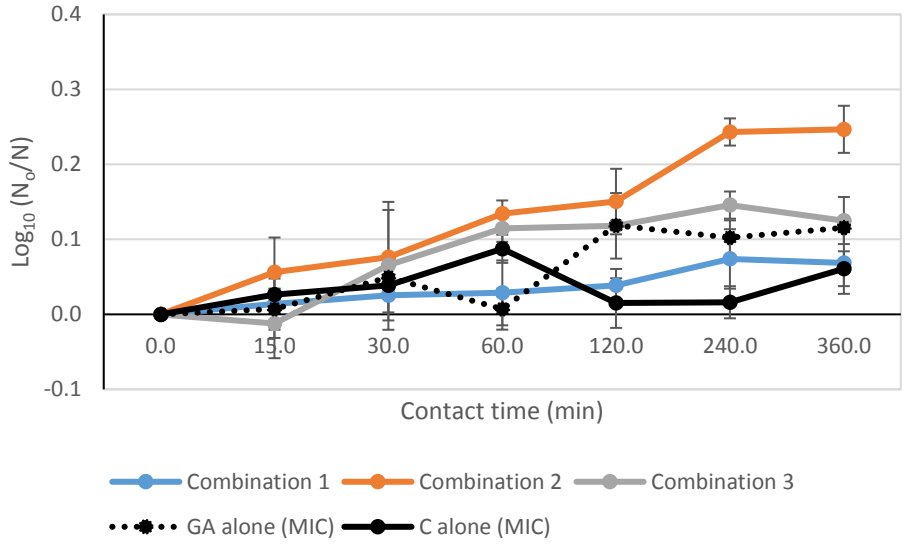



***Figure 4-26:** Combination of GA and C on S. aureus growth. Combination 1: 16 µg/ml C-4µg/ml GA; Combination 2: 8µg/ml C-8µg/ml GA; Combination 3: 4 µg/ml C-16 µg/ml GA.* The y-axis represents the log reduction of *S. aureus* population due to the treatment. The error bars represent the standard deviation obtained from three independent experiments.

## 4.4 Effectiveness of the banana peel extracts and individual phenolic compounds in reducing MS2 coliphage load

MS2, also known as male-specific coliphage, is one of the virus surrogates that can be used to study the fate of human enteroviruses. Due to its similarity to many enteroviruses, MS2 has also been used to study the efficacy of disinfection treatment (Maillard et al., 1994; Sherchan et al., 2014; Sassi, 2016). MS2 is a single-stranded RNA bacteriophage that can only infect F-pilus, found in *E. coli* bacteria (Sassi, 2016). For that reason, in most studies, *E. coli* bacteria are used as its host for propagation. The application of MS2 as a surrogate in many antiviral studies involving plant-derived compounds (Su et al., 2010) is useful as they can be propagated easily under laboratory conditions. Also, due to their ability to only infect *E. coli*, they are harmless to human health.

Using MS2 as a viral surrogate, the effects of banana extracts and selected individual compounds were evaluated by measuring their ability to reduce MS2 viral load, at an initial concentration of ~$10^6$ pfu/ml. Using *E. coli* ATCC15597 as the host, the number of surviving MS2s was determined after 24 hours of incubation at 37$^o$C. The results therefore were interpreted as a log reduction number of the untreated MS2 titer over those treated with test agents.

### 4.4.1 Antiviral activity of banana peel extract and its individual compounds

In this study, the antivirus activity of banana peel extracts was tested at two concentrations: 1000 µg/ml and 2000 µg/ml. It should be noted that the banana extracts used in this antiviral study were prepared in dimethyl sulphoxide (DMSO); preliminary results (**Appendix E2: Preliminary testing for MS2 tolerance to DMSO**) on the effects of DMSO on MS2 titer indicated that MS2 titer was gradually reduced at concentrations beyond 2000 µg/ml of the extracts, measured 10 minutes after its addition. Preliminary testing (**Appendix E1: Quality control for MS2 inactivation**) also indicated that MS2 numbers were stable at the pH used in the experiment, therefore the reduction in the MS2 titer as presented in this section was due to the interaction between the banana extract or its selected compounds and the MS2 coliphage. These results were consistent with the findings of Feng et al (2003), which indicate that MS2 in general were stable when tested at pH 6-8, in all temperatures used in their study (5$^o$, 15$^o$, 25$^o$ and 35$^o$C).

As illustrated in **Figure 4-27**, limited inhibitory activity (i.e. less than 0.1-log) on MS2 reduction was observed on both extracts at the concentrations tested. However, more linear but slightly lower inactivation in MS2 titer was evidenced at 2000 µg/ml of banana peel extracts, which indicate that treatment at higher dose is more effective in reducing MS2. But, since at a concentration above 2000 µg/ml significant reduction in MS2 number was noted because of the DMSO, the treatment was not continued further. Also, no statistically significant differences were noted between the untreated MS2 and those treated with the extracts ($p>0.05$) and increasing the treatment time up to 1 hour showed no remarkable improvement in the antiviral activity. Although currently no studies on the antiviral properties of banana extracts have been carried out, the results obtained through this study are consistent with some other studies using different plant extracts. As observed in Su et al. (2010) under a similar experimental set-up and with the same pH conditions as used in our study (~pH 7-7.4), low inhibitions of MS2 by cranberry juice (CJ) extracts and its individual compounds proanthocyanidins (PAC) were observed after a 1-hour disinfection treatment with 0.39- and 0.55-log reduction for CJ (at 100% concentrated juice) and PAC (at 0.15 mg/ml), respectively. It should also be noted that PAC and catechins (EC and C, the two major compounds in this extracts) are from the same flavanol group, and therefore it is likely that these compounds exhibit their inhibitory activity in a similar manner. Among other virus species, MS2 is also recognised as the most resistant virus species compared to other virus surrogates when tested on plant extracts, following the order of FCV>MNV-1> ϕX-174>MS2 (Su et al., 2010). Moreover, addition of phenol at concentration of 0.2% also was shown to have negligible effect on MS2 titer (initial titer of $10^9$-$10^{10}$ pfu/ml) compared to other commonly used biocides, e.g. glutaryldehyde and peracetic acid (Maillard et al., 1994), which may therefore suggest that polyphenols are not effective against MS2. The mechanism of MS2 inactivation is still not fully understood.

Among all the banana peel compounds tested, GA was found to be the most effective in reducing the MS2 viral load compared to C and EC ($p>0.05$; for GA vs C and GA vs EC), following the order of GA>C>EC. EC was found to be the weakest MS2 inhibitor among all the compounds tested. The results regarding C and EC's response to the MS2 titer were consistent with a previous study on the influenza virus. The study suggested that C is a better virus inhibitor, showing a 20% reduction in virus titer at 600 μM of catechin dose after 2-3 days of incubation, whereas no antiviral effect was observed for EC even at 1 mM concentration (Song et al., 2005).

Reductions in GA efficacy were noted as the treatment was continued beyond 40 minutes, and this was observed in all GA doses (**Figure 4-28**). An increase in the GA dose from 1mM to 2mM and 2mM to 5mM did not statistically improve the disinfection treatment in any contact times ($p>0.05$). Despite the low MS2 inactivation activity observed with GA, it should be emphasised that the GA antiviral activity observed in the present study was higher than the results reported by (Su and D'Souza, 2012), where log inactivations of MS2 were recorded at 0.04, 0.02 and 0.01 logs for GA doses of 0.1, 0.2, and 0.4 mg/ml, respectively – although treatment was conducted for 2 hours. Nonetheless, when all three concentrations of GA were tested with Feline calicivirus (FCV-F9), significant reductions in viral titer were evidenced (2.50-, 2.36-, and 0.86-log PFU/ml, respectively), with higher FCV-F9 inactivation being observed at low doses of GA (Su and D'Souza, 2012). Therefore, higher inactivation on other enteric viruses should be expected when GA is used at the concentrations employed in this study.

As illustrated in **Figure 4-29**, the exposure of MS2 to 1mM catechin (C) resulted in negligible inactivation. Similar to GA, increasing the C dose from 1mM to 2mM did not statistically improve the treatment ($p>0.05$). However, increases in C dose from 1 mM to 5.5 mM statistically reduced the number of MS2 ($p<0.05$) with about 0.1-log reduction (20% reduction) being observed at 1 hour contact time compared to less than 0.05-log (10% reduction) in 1 mM and 2mM of C concentration.

Treatment of MS2 with EC in two of the concentrations (i.e 1mM and 2mM), as illustrated in **Figure 4-30**, showed an increase in the virus's infectivity of the *E. coli* host, with a greater number of virus plaques evidenced in the treated sample compared to the control, at 20 and 30 minutes of contact

times. However, at higher dose i.e 5.5 mM, EC did not display any increases in MS2 plaque titer, but the inactivation activity was limited with 0.10-log reduction (20% reduction) after 60 minutes' disinfection.

Overall, it can be concluded that banana peel extracts and their individual compounds have only limited inactivation toward MS2 titer. When compared to the untreated MS2, only GA gives significant reduction in viral titer, ($p<0.05$ in all doses), but for EC and C, only at 5.5 mM concentration, can significant reduction in MS2 number be observed ($p<0.05$).

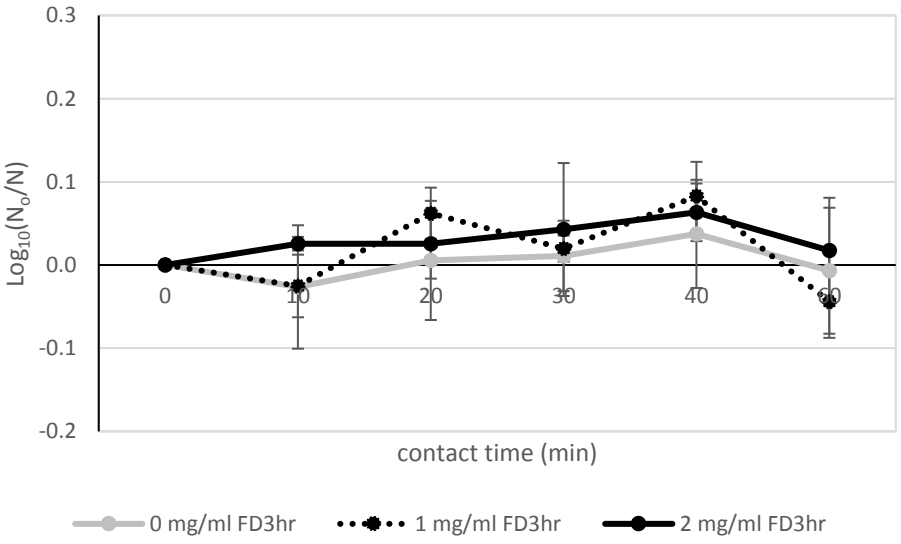



***Figure 4-27:*** *The effect of banana peel extract to MS2 titer.* The MS2 coliphage were treated with 1 mg/ml and 2 mg/ml of banana peel extract. The log reduction of MS2 titer due to the treatment (represented by the y-axis) was determined by dividing the log number of MS2 population before the treatment (at time 0) by the log number of MS2 remaining at the indicated contact time. The results shown are based on three independent experiments. Neither extract concentration achieved a statistically reduction compared to the untreated control (0 mg/ml FD3hr) ($p>0.05$)

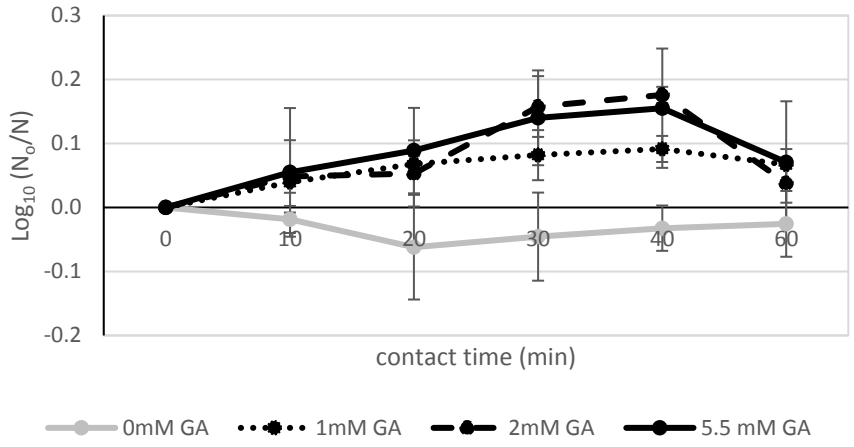



***Figure 4-28:*** *Effect of GA on MS2 titer.* The MS2 coliphage were treated with three doses (1mM, 2mM and 5.5mM) of GA. The log reduction of MS2 titer due to the treatment (represented by the y-axis) was determined by dividing the log number of MS2 population before the treatment (at time 0) by the log number of MS2 at the indicated contact time. All of GA doses, statistically reduced the number of MS2 titer ($p<0.05$) compared to the untreated control (0 mM GA). The results shown are based on three independent experiments.

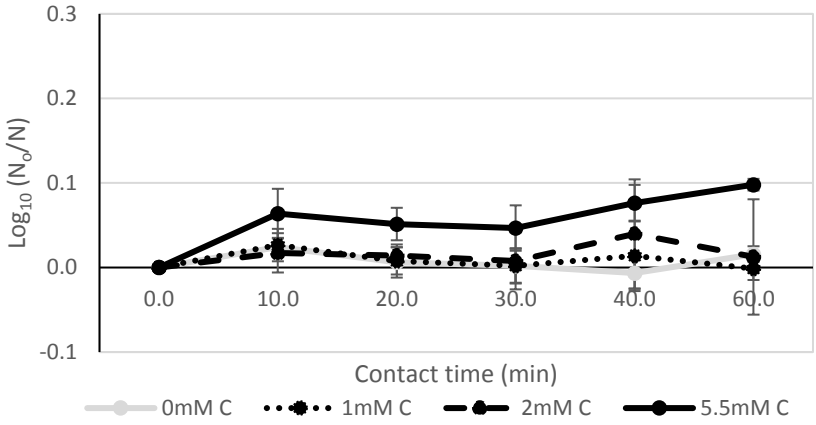



***Figure 4-29:*** *Effect of C on MS2 titer.* The MS2 coliphage were treated with three doses (1mM, 2mM and 5.5mM) of C. The log reduction of MS2 titer due to the treatment (represented by the y-axis) was determined by dividing the log number of MS2 population before the treatment (at time 0) by the log number of MS2 at the indicated contact time. Only at 5mM, C statistically reduced the number of MS2 titer ($p<0.05$) compared to the untreated control (0 mM C). The results shown are based on three independent experiments.

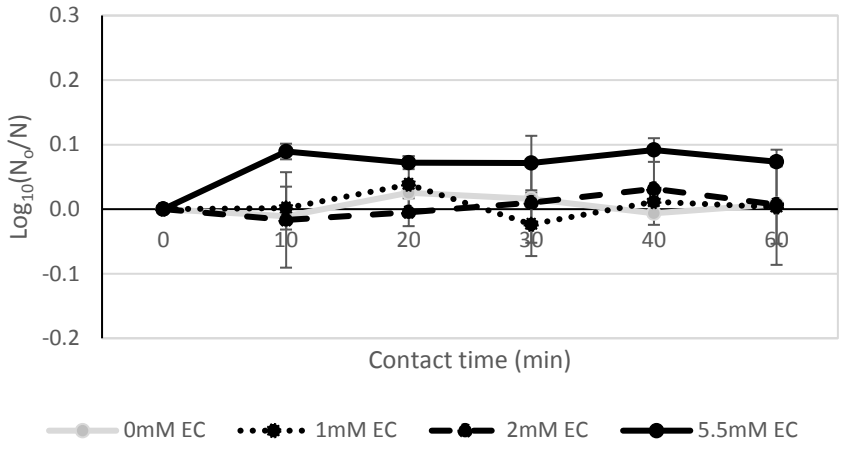



***Figure 4-30:*** *Effect of EC on MS2 titer.* The MS2 coliphage were treated with three doses (1mM, 2mM and 5.5mM) of EC. The log reduction of MS2 titer due to the treatment (represented by the y-axis) was determined by dividing the log number of MS2 population before the treatment (at time 0) by the log number of MS2 at the indicated contact time. Only at 5mM, EC statistically reduced the number of MS2 titer ($p<0.05$) compared to the untreated control (0 mM EC). The results shown are based on three independent experiments.

### 4.4.2 Antivirus study of epigallocatechin gallate (EGCg) on MS2 titer reduction

Compared to banana extracts and their individual compounds, treatment of MS2 with EGCg resulted in higher MS2 inactivation at all concentrations tested (**Figure 4-31**). Gradual increases in MS2 logs inactivation were observed after 20 minutes of contact time when it was treated with 1mM of EGCg solution, with an approximate value of 0.20-log reduction (~40% reduction) after 1 hour of the treatment. At the higher concentrations, i.e, 2 mM and 5.5 mM, slightly higher log inactivation was noted after 10 minutes of contact time, with log reduction ranging from 0.05-0.15 log (10-30% reduction), but the effect was not significant for 2 mM ($p>0.05$, when compared to 1 mM). Treatment of MS2 with 5 mM EGCg gave a higher log reduction during the first 30 minutes ($p<0.05$, when compared to 1 mM), though a reduction in EGCg efficacy was noted when the treatment was carried out beyond 30 minutes. This may be due to the fact that at a higher EGCg dose, the viral species tends to form aggregates, which may then limit the effectiveness of the treatment over time. A similar phenomenon was evidenced in Su et al. (2010). Overall, poor virucidal activity was exhibited by EGCg when tested on MS2 coliphage. When compared to the untreated control, only at higher concentrations, i.e. 2 mM and 5.5 mM, was a significant reduction in MS2 titer seen ($p<0.05$)

Previous studies on EGCg against other virus species have been extensively reviewed by Steinmann et al. (2013). EGCg is recognised as an active inhibitor against human enteroviruses such as the hepatitis C virus (HCV) (Calland et al., 2012; Wang et al., 2016), the herpes simplex virus type 2 (HSV-2) (Isaacs et al., 2008), and the human immunodeficiency virus (HIV) (Weber et al., 2003). Although limited activity on MS2 was observed in the present study, it should be noted that MS2 is a surrogate small non-enveloped virus, whereas all of the above-mentioned human viruses consist of enveloped viruses. Enveloped viruses in general consist of a protected protein coat (capsid) covered by a viral envelope made from glycoproteins, which are responsible for binding to host cells. As observed in the *S. aureus* experiments, it is well accepted that EGCg in general has higher affinity for binding to a variety of proteins particularly peptides, therefore it is likely that EGCg exhibited its antivirus activity by binding to the glycoprotein envelopes, thus preventing the attachment of the virus species to the host cells. Since the MS2 species lacks a glycoprotein envelope, it is possible that the absence of the target sites prevented EGCg from effectively reducing MS2 titer, as observed in the current study. In fact, the

production of macromolar glycoprotein complexes has been evidenced after treating HSV with EGCg and complete inhibition (≥4-log reduction) was observed after 20 minutes at a 50µM EGCg dose (Isaacs et al., 2008). Meanwhile, in this study, at a concentration 100 times higher than that used in Isaacs et al. (2008), only up to 0.15-log reduction was observed after 30 minutes of treatment. Nevertheless, the effects of EGCg on the non-enveloped adenovirus showed that EGCg was able to delay the virus's infection of the host cells, although significant inhibitory activity towards the virus particle can only be seen after 18 hours of treatment; with more than 1-log reduction being recorded at 100 µM EGCg (Weber et al., 2003). This therefore explains the lower sensitivity of EGCg during the one hour testing, as observed in the present study.

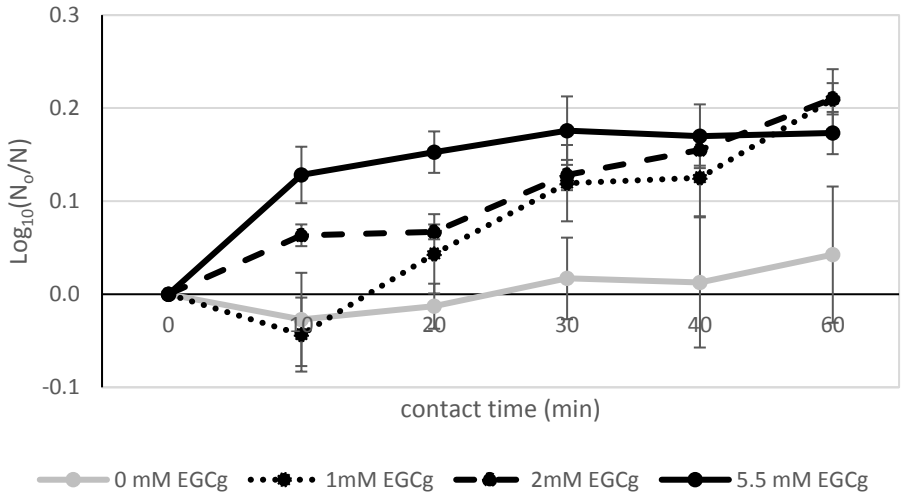



***Figure 4-31:*** *Effect of EGCg on MS2 titer.* The MS2 coliphage were treated with three doses (1mM, 2mM and 5.5mM) of EGCg. The log reduction of MS2 titer due to the treatment (represented by the y-axis) was determined by dividing the log number of MS2 population before the treatment (at time 0) by the log number of MS2 at the indicated contact time. Only at 2mM and 5mM, EGCg statistically reduced the number of MS2 titer ($p<0.05$) compared to the untreated control (0 mM EGCg). The results shown are based on three independent experiments.

## 4.5 Hydrogen peroxide generation and its correlation to observed antimicrobial activity

The generation of hydrogen peroxide has been proposed to be one of the mechanisms involved in phenolic compounds' antimicrobial activity (Kajiya et al., 2001; Nakayama et al., 2002; Akagawa et al., 2003; Arakawa et al., 2004). However, currently there is no evidence to suggest that the hydrogen peroxide generated by compounds could interfere with viral inactivation. A study on hydrogen peroxide activity against MS2 has been carried out by Asghari (1993). The results of the study indicate that bacteriophages (including MS2) in general are more resistant to hydrogen peroxide treatment compared to *E. coli*. Their study also suggest that generation of OH radicals from hydrogen peroxide was not the mode of action in virus inactivation. Moreover, the addition of a biocide solution containing 0.5-2% of hydrogen peroxide has been shown to cause only 0.06-log inactivation in the MS2 population (Sassi, 2016), suggesting that hydrogen peroxide has negligible effect on the MS2 titer. Currently, the mode of inactivation for MS2 is not fully understood.

In this study, the formation of hydrogen peroxide was measured for 6 hours at three different concentrations – 65, 125 and 250 μg/ml – which were in the range of MIC for some of the compounds involved. Similarly, the amount of endogenous hydrogen peroxide generated by the extracts at different extraction times was also measured, to understand whether the variation in extract inhibitory activity (observed through their MIC value) was also related to hydrogen peroxide formation.

**Figure 4-32** shows the measured hydrogen peroxide in all of the banana extracts. As can be seen from the graph, the amounts of hydrogen peroxide generated by the extracts are in the range of 20 to 33 μM, 2 to 6 hours after extract addition. However, there was no significant difference between the values for all of the extracts ($p>0.05$), which may suggest that the composition of the active phenolic compounds in the extracts has a direct effect on their antibacterial activity, more so than from the formation of hydrogen peroxide. As observed earlier in **Figure 4-5** and **Figure 4-6**, a higher composition of phenolic compounds in extracts from 3 hours' maceration was consistent with the MIC data measured for the extracts, for both bacterial species in this study.

**Figure 4-33** presents the amounts of hydrogen peroxide measured from the selected individual phenolic compounds. As can be seen, increases in hydrogen peroxide were observed with an increased dose of all compounds. Among all the compounds tested, EGCg and GA were found to generate higher average amounts of hydrogen peroxide compared to C and EC, with the hydrogen peroxide value was recorded ranging from 30-50 μM for EGCg, and 40-60 μM for GA at equal concentration of 250 μg/ml. Based from the results, it can be seen at an equal concentration of 250 μg/ml, GA generated slightly higher amounts of hydrogen peroxide than EGCg. This result suggests that if hydrogen peroxide is the main mechanism involved in the antimicrobial activity, higher inhibitions of the bacteria growth should be observed in the treatment with GA. However, in fact the opposite was observed. As illustrated in the interaction of *S. aureus* in **Figure 4-11** and **Figure 4-12** for EGCg and GA, respectively, extremely higher inhibitions (i.e. almost 5-log) were noted when EGCg at 250 μg/ml was added, in terms of *S. aureus* growth. Meanwhile, less than 0.5-log was seen resulting from GA addition– this result therefore suggests that other mechanisms may be involved in the EGCg-*S. aureus* interaction, apart from hydrogen peroxide generation, such as potential direct action of the compound against vital components of the bacterial cells, e.g. peptidoglycan, as proposed by Yoda et al. (2004).

In order to confirm this hypothesis, the disinfection study of EGCg on *S. aureus* was repeated for one hour at its MIC value (**Figure 4-34**), with the addition of catalase (~10 units/ml) to inhibit the generation of hydrogen peroxide formed by EGCg. The addition of catalase (~10 units/ml) to the control did not cause any inhibition of *S. aureus* cells. As predicted, a slightly higher inhibition of *S. aureus* growth was achieved with the addition of catalase, which is probably because the addition of catalase may also inhibit the generation of defence protein by *S. aureus* cells (Amin and Olson, 1968), thus making the cells more sensitive to EGCg interaction. This observation therefore suggest that the higher inhibitory activity observed in the *S. aureus* experiment with EGCg was due to other mechanisms i.e. attachment on the peptidoglycan, and not because of hydrogen peroxide. Indeed, microscopy observation on *S. aureus* cells also indicates that the addition of EGCg to *S. aureus* (ATCC 25923) cells results in permanent changes to *S. aureus* cells, and the addition of catalase does not offset the cell morphology changes (Cui et al., 2012). In the same study, the authors also demonstrated the effects of EGCg on *E. coli* cells was due to hydrogen peroxide; when catalase was added to the solution, the

complete recovery of *E. coli* cells was achieved. As observed in this study, higher inhibitions in *E. coli* was noted after 4 hours of EGCg contact (**Figure 4-16**), and this was consistent with the amounts of hydrogen peroxide formation, as seen in, **Figure 4-33**(a). This results therefore indicate that interaction of EGCg to *E. coli* may possibly due to hydrogen peroxide, which would agree with a previous report by Cui et al. (2012).

The results by Cui et al. (2012) also suggest that hydrogen peroxide had a temporary effect on bacteria cells, as recovery of the cells can be observed a few hours after the treatment. If hydrogen peroxide was deemed to be the mechanism in involved in inhibitory activity of banana peel-derived compounds, this may therefore explain the fluctuations in log activity observed in the compounds towards both of the bacterial species. It is however, unclear whether the inhibitory activity exhibited by the compounds was due to hydrogen peroxide or not, since the log inhibitory activity was too low (i.e. less than 0.5-log in all of the compounds) for significant changes in bacterial population to be observed. It is worth noting that at least milimolar concentrations of hydrogen peroxide are needed to inhibit bacterial growth (Koivunen and Heinonen-Tanski, 2005; Imlay, 2008). Furthermore, as reported by Nakamura and Niwano (2017), at concentration of 500 mM hydrogen peroxide, only 1-log reduction in *S. aureus* is seen.

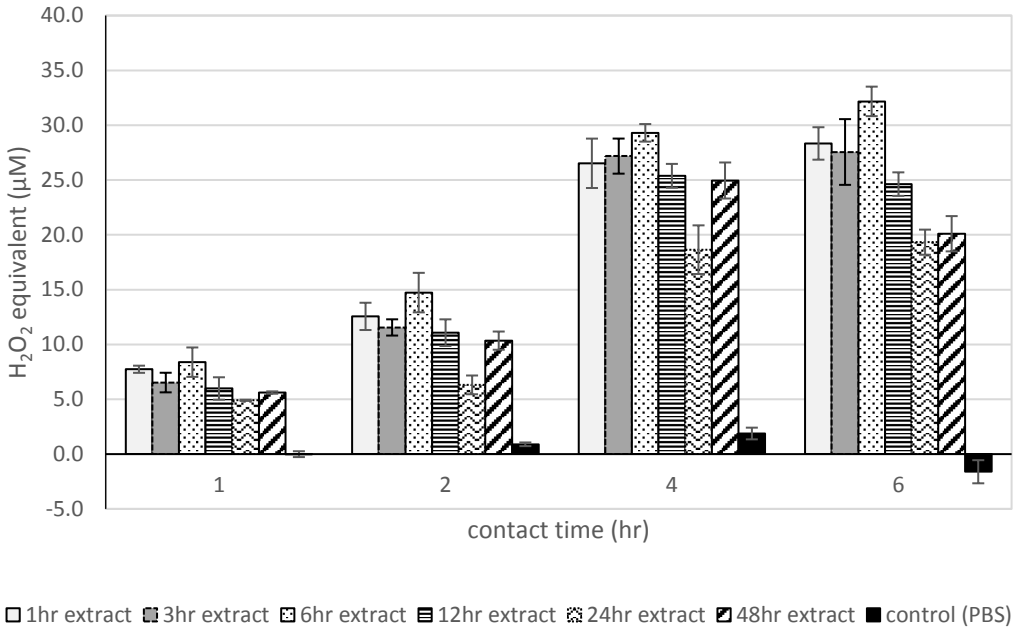
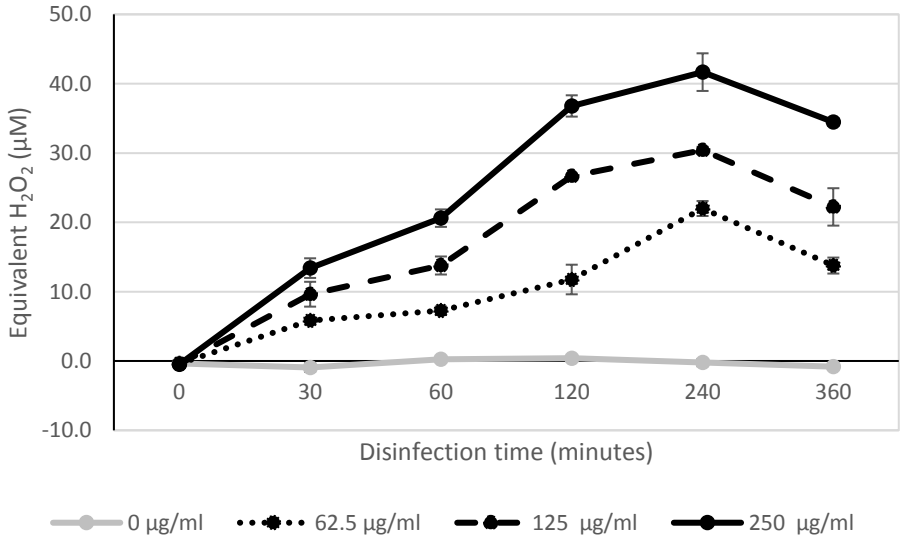
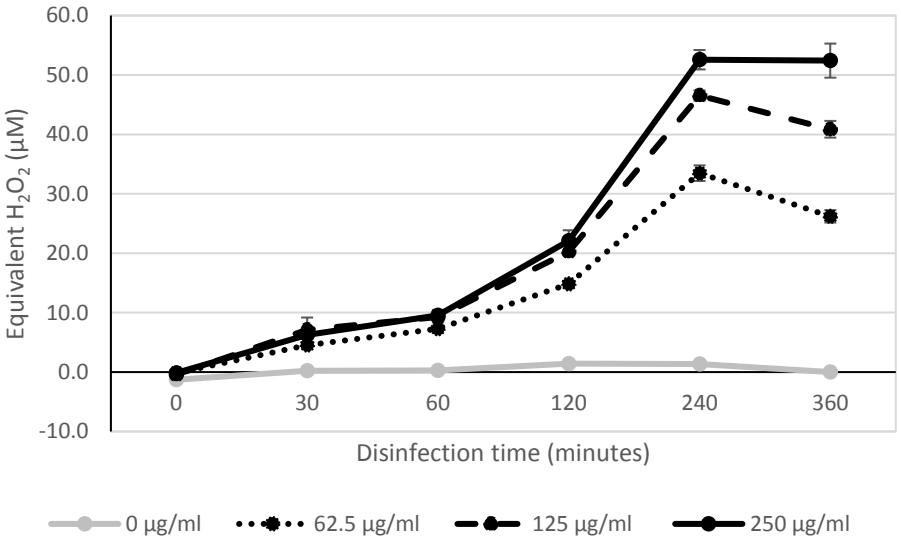


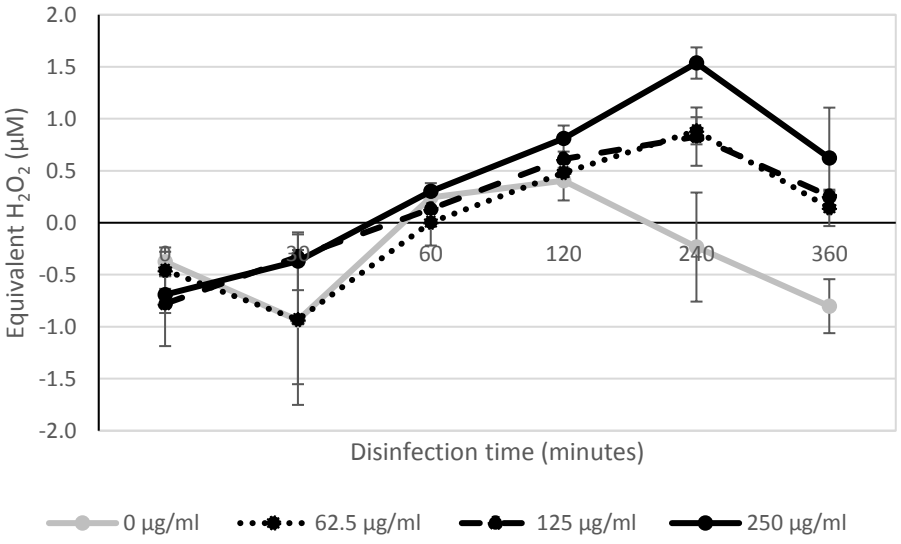

***Figure 4-32:*** *Generation of hydrogen peroxide in the freeze-dried banana extracts, from different extraction times.* All of the extracts were tested at an equal concentration of 2 mg/ml, and no bacteria was added to the PBS during the experiment. Results shown are based on three independent experiments.



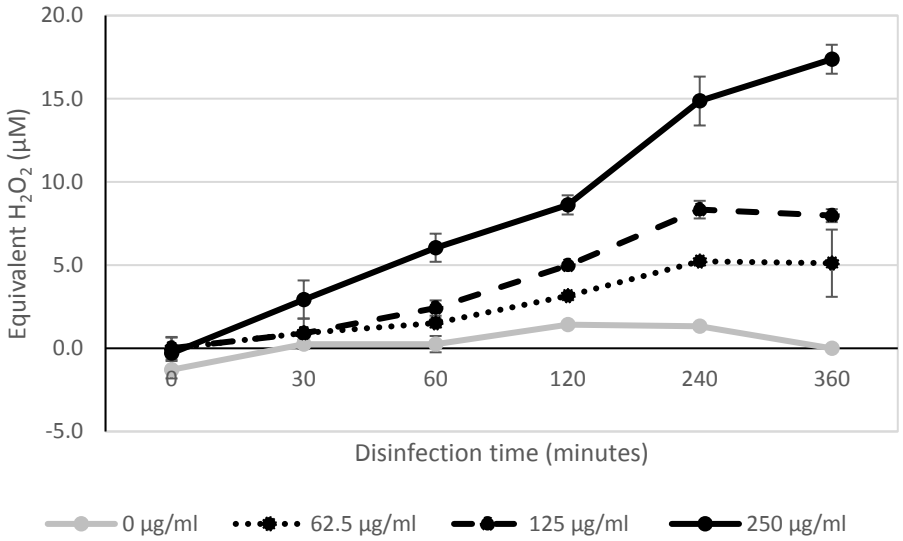

(a)


60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
Equivalent $H_2O_2$ (µM)
0
30
60
120
240
360
Disinfection time (minutes)
0 µg/ml
62.5 µg/ml
125 µg/ml
250 µg/ml

(b)


2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Equivalent $H_2O_2$ (µM)
30
60
120
240
360
Disinfection time (minutes)
0 µg/ml
62.5 µg/ml
125 µg/ml
250 µg/ml

(c)




(d)

***Figure 4-33:*** *Hydrogen peroxide generation in (a) EGCg (b) GA (c) C (d) EC, measured at three concentrations: 62.5, 125 and 250 µg/ml.* Results shown are based on three independent experiments.

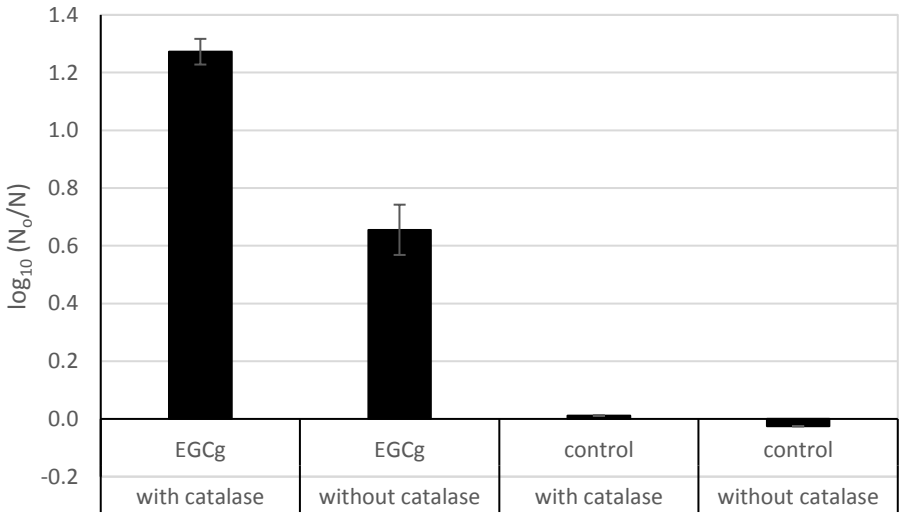



***Figure 4-34:*** *Comparison of S. aureus log inhibition using EGCg at 62.5 µg/ml concentration, after 1 hour contact time.* Catalase was added at 10 units/ml concentration. The results shown are based on three independent experiments.

# Chapter 5 **DISCUSSION**

A biocide is any compound/substance used to inhibit the growth of bacteria. While the application of synthetic chemicals (e.g. chlorine) as biocide agents has been shown to effectively reduce the transmission of pathogenic diseases, their applications can have drawbacks. Hence, efforts have been made by researchers to search for an alternative to synthetic chemical biocides. Plants have been proposed as an ideal candidate for biocides based on their proven therapeutic ability for human health. If a biocide could be derived from a plant-related waste (e.g. food waste) and thereby derive value from the waste material that would be even better. This study therefore aimed to test the efficacy of plant-derived compounds from banana peels extracts, as a potential biocide, in the treatment of three target surrogate organisms, namely *S. aureus*, *E. coli* and MS2 coliphage.

In this study, banana peel was chosen as the raw material in studying the derived antimicrobial compounds from plants. The selection of this agricultural by-product is made based on the significant role of banana in food commodity and food security, especially to its producing countries which mainly consisted of developing countries. Moreover, bananas are the most consumed fruit in the European market (Del Mar Verde Mendez et al., 2003), highlighting the importance of this tropical fruit in the global market. Despite of its economic importance, consumption of banana has been shown to results in high generation of waste by-products. Banana peel, one of the by-products from the banana industry, are a rich source of phenolic compounds, some of which have been previously demonstrated to have broad spectrum activity against various bacteria species.

Banana peels extracts consisted of a high concentration of flavonoids compounds, of which some have been identified. Due to solubility of most flavonoids in organic solvents, in most cases, the extraction of banana extracts was carried out through simple extraction process using varying degree of polar solvents. However, previous study on banana extracts have shown that aqueous extracts of banana peels i.e. extracts produced in water, are ineffective in inhibiting microbial species growth (Mokbel and Hashinaga, 2005). Taking this into consideration, in this study the banana peel extracts were produced through extraction with methanol, as proposed by González-Montelongo et al. (2010). Preliminary

observation on the fractionation of banana extracts with less polar solvents also showed that none of the compounds could be separated using non-polar solvents such as hexane and chloroform, thus confirming that banana extracts used in this study consisted of polar phenolic compounds.

The antimicrobial analysis on the methanolic peel extracts was carried out by measuring the MIC of the extracts. The results indicate that *S. aureus*, a Gram-positive bacteria is more sensitive to banana peel extracts than Gram-negative bacteria, *E. coli*. The lowest MIC values were noted from extracts from 3 hours' extraction time, at 0.63 mg/ml and 1.25 mg/ml for *S. aureus* and *E. coli,* respectively. The results of this study are consistent with other studies using plant extracts (Thuille et al., 2003; Konaté et al., 2012; Ushimaru et al., 2012), which showed a higher affinity of plant extracts to Gram-positive bacteria. Previous research on individual banana peels compounds also suggested higher sensitivities of Gram-positive bacteria to flavonoids compared to Gram-negative bacteria – which is likely due to the ability of most flavonoids to form complexes with proteins (Cushnie and Lamb, 2005). The presence of an outer layer in Gram-negative bacteria has been proposed as the main reason for reduced activity in flavonoids.

One of the reasons for the extracts inhibitory activity has been proposed to be due to the presence of active antimicrobial compounds in the extracts (Vuuren et al., 2011; Negi, 2012). Using HPLC, four compounds were identified in the banana peel extracts; catechin, epicatechin, gallic acids and dopamine. The presence of these compounds has been reported previously in banana peel extracts (Kanazawa and Sakakibara, 2000; Someya et al., 2002; González-Montelongo et al., 2010; Rebello et al., 2014). Out of the four, three compounds from banana peel extracts in this study were recognised as antimicrobials. Catechin, epicatechin and gallic acids in general are common compounds that can be found in many fruit by-products, such as mango kernel and apple peel extracts (Babbar et al., 2013). The results of investigation of the polyphenol content of the extracts indicate that a higher proportion of these phenolic compounds was extracted during the first 6 hours of maceration, which is positively correlated to the MIC value of the extracts – where higher activity was noted in the extracts from 1 to 6 hours' maceration. Therefore, it was hypothesized that the antimicrobial activity exhibited by the extracts may be due to the combined activity of these individual compounds in the extracts.

Consequently, the disinfection study on each bacterial species was carried out to further explain the activity exhibited by the extracts and their individual compounds. Biocides are normally formulated at a high dose, beyond their inhibitory concentration, in order to ensure a higher degree of microbial inactivation. Although it is known that biocidal activity is normally expressed in terms of MIC values, it should be emphasised that MIC assessment is made based on long incubation (i.e. 18-24 hours) of the compounds in contact with the target bacteria species. Therefore MIC cannot be used as an indicator of the actual water or surface disinfection performance of the extracts or the compounds, where antimicrobial activity would be desirable on the order of minutes or even seconds. In this study, the efficacy of the extracts and the identified polyphenols compounds was evaluated in a series of 6-hour disinfection treatments. Although a 6-hr contact time is not considered as practical for water disinfection, this contact time was chosen in order to observe their inhibitory trends, so that application as a biocide can be established.

The results showed that banana extracts have the potential to be considered as biocides for inhibiting Gram-positive bacteria rather than Gram-negative bacteria. The addition of banana extracts to *S. aureus* at a concentration of ~1.3 mg/ml (2 x MIC) resulted in up to 0.5-log reduction (~70% reduction), though the activity was dose- and time- dependent. The interaction of the extracts with *E. coli* followed a dose-dependent manner, with increased doses resulting in higher inhibitions. However, due to the permeability barrier in the outer membrane of *E. coli*, at least 5 mg/ml (4 x MIC) of banana extracts were required for the treatment. At low doses of the extracts, an increase in the *E. coli* population (i.e. negative log reduction) was evident. The plausible explanation for this is that some compounds in plant extracts, particularly from plant residue, are known to be bounded by glycosides (sugar moiety) in their structure (Mandalari et al., 2007), which may therefore favour the growth of the *E. coli* species.

Similar to the extracts, the MICs of each identified compound were also determined in order to understand whether higher disinfection efficacy could be obtained from the individual compounds and also to determine whether the activity exhibited by the extracts was due to the singular effects of the compound, or the combination of their activity. The results indicate that all three compounds chosen had lower MICs than the extracts for *S. aureus*, but a higher MIC value was noted in two of the major

compounds (EC and C) for *E. coli*. These two major compounds were not considered as 'active antimicrobials' for *E. coli* with MIC of more than 1000 µg/ml. Therefore EGCg, a widely studied catechin compound, was included in this study. Among all the compounds tested, EGCg was shown to have higher inhibitory activity, compared to GA, EC and C, against both of the bacteria species. Higher inhibitions were noted in *S. aureus* than in *E. coli* for all the compounds used in this study. The MICs of EGCg were recorded at 62.5 and 250 µg/ml for *S. aureus* and *E. coli*, respectively. EGCg in general is formed through an esterification of GAs and catechins. Although the presence of this compound has been reported previously in bananas (Septembre-Malaterre et al., 2016), no EGCg was detected in any of the extracts produced in this study.

The antimicrobial activity of polyphenols that has been reported could be influenced by the degree of hydrophobicity of a compound (Kajiya et al., 2001). Compounds with higher lipophilic properties, compared to polar compounds, are hypothesised to be more effective against bacteria cells due to the lipid-lipid interaction (Dorman and Deans, 2000). EGCg in general had higher hydrophobicity than the other compounds tested in this study (which can be observed through their elution time in HPLC). Therefore it is expected to have a better interaction with the bacteria cells. Alkylation of a gallate moiety into the catechin structure has been proposed to increase catechins hydrophobicity, thus enhancing the degree of partitioning in cell membranes (Tsuchiya, 1999). As shown in this study, the addition of EGCg at a concentration of 125 µg/ml caused more than 1-log reduction in *S. aureus* in 30 minutes, while almost negligible activity was observed by the two other catechins (EC and C). Cushnie et al. (2008) also demonstrated that the alkylation of 3-O-acyl derivatives to EC improved the interaction of EC with *S. aureus* NCTC 6571 (the same species was used in this study), with approximately 2-log reduction after 1 hour of treatment at a concentration of 50 µg/ml. Their study also suggested that epicatechin derivatives have potent activity compared to gallated epicatechin (Cushnie et al., 2008), thus highlighting the importance of compounds hydrophobicity on antimicrobial activity.

It should be emphasised that other than its lipophilic character, EGCg was also proposed to interacts with the cells peptidoglycan membrane (Yoda et al., 2004). This explains the higher activity exhibited by this compound towards *S. aureus* compared to *E. coli*. A comparative study on EC and EGCg also revealed that EGCg caused more intensive damage to membranes compared with EC,

indicating the importance of a galloyl moiety on the antimicrobial activity of catechins (Ikigai et al., 1993). The difference between EC and C, however, has been proposed to be due to the stereospecific effects on membrane fluidity (Tsuchiya, 2001). Compound C in general is in trans-form, while EC is in cis-form. It has been demonstrated that catechins in cis form were more effective for reducing membrane fluidity than catechins in trans form (Tsuchiya, 2001).

The other compound that was studied was gallic acid (GA). Various studies have reported that GA is an active inhibitor of various bacterial species (Borges et al., 2013; Diaz-Gomez et al., 2014; Pinho et al., 2015). However, the present study showed that GA on its own may have limited application as a biocide agent. Low inhibitory activity of GA with maximum ~0.3-log reduction (50% reduction) was noted in *S. aureus* after 6 hours of the treatment, and even less was observed in *E. coli*. This study also showed that GA exhibits inconsistent inhibitory activity towards *S. aureus* with increases and decreases in its activity were observed under prolonged incubations with the bacteria. The fluctuations in GA activity were explained by Pinho et al. (2015), who believed that although GA has antimicrobial properties, it can be easily oxidised at certain pH values. Weak acids such as GA in general interact with microorganisms through an undissociated form of its ions. Gallic acid in particular has two ionisation moieties: the carboxylic and hydroxyl groups, which are attached to its phenolic ring. Gallic acid was hypothesised to be in its undissociated form at pHs less than 4.4, while at pH 5 to 7, it contains a deprotonated carboxylate group. At a basic pH, both the carboxylic and hydroxyl group are deprotonated (Pinho et al., 2015). It is important to emphasise that, although GA was a weak inhibitor of both bacterial species under experimental procedure in this study, it was shown to works in synergy with the other compounds included in this study, and also has been reported to prevent the formation of biofilms in bacteria populations (Borges et al., 2012), which may therefore suggest their practicality in water applications (e.g. as a disinfectant for storage tanks) if its limitation as mentioned earlier could be overcome in the future. Indeed, combination of GA with UV-A light was shown to cause significant damage to *E. coli* cells' membranes, and inhibits the formation of superoxide dismutase (SOD), a protein responsible for cellular defences (Wang et al., 2017).

Lower inhibitions were noted in *E. coli* species, for all individual compounds, including the extracts. *E. coli* in general is a Gram-negative bacteria, with an additional negatively charged outer

membrane known as lipopolysaccharide (LPS). As shown in many disinfections studies, Gram-negative bacteria in general have higher resistance to many antimicrobial agents because this outer membrane works as a permeability barrier and prevents the interaction between antimicrobials and bacteria cells. In a study by Ikigai et al. (1993), the authors demonstrated that the resistivity of Gram-negative bacteria to EGCg was due to the negative charge of the LPS membrane. In addition, high tolerance of gram negative towards GA has been proposed due to the activation of protein superoxide dismutase (SOD), resulting from the formation and generation of hydrogen peroxide by the compound (Wang et al., 2017). In this study, the effect of EGCg and GA was evaluated, in addition to other Gram-negative bacteria, and the results indicate a similar weak inhibitory effect (i.e. < 1-log) in all of the species used in this study. This study also demonstrated that the weak inhibition shown by EGCg was not specific to the *E. coli* species, but to all Gram-negative bacteria. This therefore suggests that the application of natural compounds such as EGCg and GA as biocides agents for water disinfection may be restricted, as most pathogenic bacteria are gram negative.

Overall, as observed in the activity of individual compounds in banana extracts, it can be concluded that slightly higher inhibitions on *S. aureus* were noted in the extracts, compared to the pure polyphenol compounds. The results of this study suggest that the inhibitory effects of extracts may not only be due to the presence of GA, EC and C in the extracts, but also the complex biochemical interaction between the compounds and the target organisms, many of which are still unidentified. Nevertheless, the combination of GA-EC and GA-C was shown to enhance the inhibitory activity of the compounds in *S. aureus*. In contrast, there were no obvious interactions between the compounds in the extracts and *E. coli* inhibitions, as when the compounds were combined, no synergy was observed.

In this study, the efficacy of the extracts and their individual compounds as virucides was also evaluated using bacteriophage MS2 as a model organism. MS2 is used in many disinfection studies due to the fact that it exhibits similar morphology and is relatively resistant to disinfection treatments compared to human pathogenic viruses. Although the specific mechanism that is involved in virus inactivation is still unknown, studies on virus inactivation have shown that MS2 bacteriophage in general are more resistant to other virus surrogates such as FCV and ϕ-X174 (Maillard et al., 1994; D'Souza and Su, 2010). A previous study on MS2 inactivation by Su et al. (2010) demonstrated limited

inactivation on MS2 using cranberry juice at pH 7 with 0.39-log reduction (60% reduction) after 1-hour treatment. However, when the pH of the extracts was adjusted to 3, an increase in log inactivation was evident (1.14- log). Their study therefore suggests that pH has a profound effect on the infectious ability of MS2. MS2 in general has an isoelectric point (pI) of 3.4, and at pH values near to its pI the aggregation of organic matter to its virus particle occurs. Therefore, it is likely that at pH 3, as used by Su et al. (2010), the virus particle was compromised due to the aggregation of compounds with antimicrobial activity, thus reducing the ability of the phage to infect its host, and subsequently leading to an increase in log inactivation. However, at a neutral pH, repulsion between the compounds and phage occurs, hence no remarkable difference in phage infectivity can be observed. No notable differences between the treated phage and the control, in the extracts and also in the individual compounds, were observed after the treatment. It should be noted that in this study, the disinfection of MS2 phage was carried out at pH 7.2-7.4. It can be concluded that under the disinfection settings, that the extracts and individual compounds were not effective as virucides due to their inability to reduce the MS2 viral load. Among all the compounds tested, EGCg was found to be best at reducing the MS2 viral load, with maximum inactivation of 0.2-log (40% reduction) noted after 1 hour treatment at 1mM EGCg dose.

Hydrogen peroxide has been proposed as one of the mechanisms involved in the biological activity of plant compounds. The generation of hydrogen peroxide has been suggested to occur as a result of auto-oxidation of polyphenols, particularly at pH 7-9 (Akagawa et al., 2003). In order to understand the effects of hydrogen peroxide generated on the activity observed in this study, the concentration of hydrogen peroxide generated was quantified. Among all the compounds studied, GA and EGCg were shown to produce higher amounts of hydrogen peroxide compared to the other two catechins. The results also indicate that at an equal concentration of GA and EGCg, slightly higher levels of hydrogen peroxide were generated by GA compared to EGCg. This result however seems to contradict with the inhibitory activity exhibited by the compounds on both of the tested bacterial species, as higher activity was observed by EGCg compared to GA, with more pronounced inactivation noted against *S. aureus*. Moreover, addition of catalase into the *S. aureus* cells showed that higher inactivation can be achieved after 1-hour contact time.

This result therefore confirms that higher inhibitory activity as observed in the EGCg-*S. aureus* treatment, is not due to hydrogen peroxide formation but more likely due to interaction of the compound with vital cell components (e.g. peptidoglycan) as proposed by other researchers (Yoda et al., 2004; Cui et al., 2012). While hydrogen peroxide generation may be one of the mechanisms involved in the other compounds (i.e. GA, EC and C) inhibitory activity as proposed by other researchers (Nakayama et al., 2002; Akagawa et al., 2003; Arakawa et al., 2004), the results from this study showed that under the conditions and concentrations used in the disinfection study, the amounts of hydrogen peroxide generated were too low for significant effects on bacterial growth to be observed. At least millimolar concentrations are required before significant reduction in bacteria viability can be observed (Koivunen and Heinonen-Tanski, 2005; Imlay, 2008), whereas much lower hydrogen peroxide concentrations were generated in this study. This may therefore explains weak inhibitory activity (i.e. less than 1-log reduction) exerted by the compounds during the disinfection.

Overall, this research was the first to observe the inhibitory activity of banana peel extract-derived compounds as mixtures and as isolated compounds, against three different target organisms. The findings suggest that banana peel extracts do have potential as a biocide, with their activity most pronounced against Gram-positive bacteria. In terms of overall activity, the extracts have higher activity than their individual compounds. Among all the individual compounds used, EGCg was shown to have the most potential to be further explored as a biocide, having achieved the highest log reductions against all the tested target organisms. Recent attempts to exploit the antimicrobial ability of EGCg as a hand sanitizer have been reported recently by Zhang et al. (2016). With some modification of the EGCg molecular structure, it was demonstrated that lipophilic EGCg was able to be used as hand sanitizer with log-inactivation up to 6-log against poliovirus (a non-enveloped virus) compared to other commercialised hand sanitizer. Their study indirectly proved that, with slight improvements in EGCg hydrophobicity character, application of EGCg as an effective biocide agent for pathogenic microorganism including viruses can be warranted. Future studies however should also include a detailed assessment on its toxicity and safety, so that its safe application as a biocide can be established.

The results of the present study also suggest that the combined activity of two banana peel extract-derived compounds is an option for improving the disinfection performance, with gallic acid, a weak inhibitor on its own, playing an important synergistic role when applied with catechins.

Finally, however, the banana peel extracts and their individual compounds may have limited application as a broad-spectrum biocide due to their inability to effectively reduce viral species.

Further research on plant-derived biocides may address the following issues:

- It should be noted that, only three compounds from the banana peel extracts were considered. It would be useful if most of the compounds available in the extracts can be quantified in order to understand the overall activity exhibited by the extracts. As demonstrated, the addition of GA to two catechin compounds indicates that there is synergistic interaction between the compounds, suggesting that other compound interactions may also enhance (or impede) the overall inhibitory activity of the mixtures.
- While the application of polar compounds may have benefits in term of their application in water disinfection treatment, as shown in this study, polar compounds (i.e. GA versus EGCg) have relatively lower inhibitory activity against bacterial cells. Therefore, further study into natural plant antimicrobials, especially those meant for water treatment applications, may consider the addition of a surfactant, which may enhance the interaction between the bacterial cells and the compounds. For instance, the addition of an anionic surfactant such as sodium lauryl sulfate into cranberry and grape seed extracts has been shown to increase the log inhibitory activity of the extracts and also reduce the contact time required for effective inactivation of microbial species (Weinsetel, 2006).
- The study showed that EGCg is an effective inhibitor for reducing *S. aureus* populations. However, it should be noted that this study was carried out without considering 'dirty' environments (e.g. with particles or other potentially interfering constituents present), which may reduce the efficacy of the compound. As it is known that *S. aureus* can be found on most surfaces, future studies considering this compound as a biocide should also consider potential interfering factors. For instance, bovine albumin (3.0 g/L) can be used to simulate dirty

conditions, in accordance with the requirement made by European Standards to validate disinfectant tests (Ríos-Castillo et al., 2017).

- The study also showed that MS2 inactivation was not effective at pH more than 7, therefore further study on the activity of natural biocides, particularly on viral species, could also consider the pH as one of the important factor in the disinfection assessment, as increase in activity was noted by other researchers with the reduction of pH (Su et al., 2010). It is also recommended that the extract compounds be tested against other virus types, given that MS2 may be more resistant than others.
- Before the extract compounds studied in this research could be developed into commercial products (e.g. household cleaners or water disinfectant solutions), further research would be needed into the availability and geographic distribution of the banana peel waste and the logistical challenges of scaling up the biocide production, to ensure that an affordable and attractive product could be generated and brought to market successfully. A further commercialisation study focused especially on EGCg is warranted on the basis of the promising potential that it demonstrated in this study, although it may need to be combined with other compounds (perhaps especially in combination with GA), to provide synergistic benefits in terms of the inactivation of a wide range of bacteria and viruses, depending on the intended application/market.

# Chapter 6 CONCLUSIONS

The most important original contributions to knowledge that arose from this research are the following:

(1) The three compounds found in banana peel extracts at the highest concentrations and possessing the most antimicrobial activity are catechin, epicatechin and gallic acid. Catechin and epicatechin are the predominant compounds in all extracts. A higher inhibitory activity was noted in the extracts obtained within the first 6 hours of extraction time. Increasing the extraction time, increased the amounts of extractable solids obtained but caused a significant reduction in the antimicrobial activity of the extracts. This suggests that longer extraction times should not be considered for future antimicrobial studies using this material.

(2) Higher inhibitory activity was exhibited by banana peel extracts towards *S. aureus* bacteria, while weaker activity was observed against *E. coli*. This implies that the application of the extracts is limited to inhibiting Gram-positive bacteria only and their application for water disinfection specifically may be restricted, as most waterborne pathogenic bacteria are Gram-negative.

(3) The antimicrobial activity of the extracts was proportional to the concentrations of the individual antimicrobial compounds available in the extracts, indicating that the inhibitory activity of the extracts is generated through a combination of effects from their individual compounds.

(4) Among all individual compounds tested in this research, EGCg is the most promising inhibitor for both *S. aureus* and *E. coli*. All of the individual compounds tested had higher inhibitory effects against *S. aureus* than against *E. coli*.

(5) Combining two compounds, i.e. EGCg-GA, EC-GA, C-GA, improves the disinfection performance of the biocides in inhibiting *S. aureus* growth. This also proves that the activity exhibited by the extracts was indeed through different complementary physico-chemical interactions between each compound and bacterial cells. It should be emphasised that, although GA on its own is a weak inhibitor, due to its ability to interact with cell hydrophobicity, higher

inhibitions were observed when GA was used alongside other compounds. Nonetheless, this observed synergistic interaction is limited to *S. aureus*, as no synergy was observed in the case of *E. coli*. It can be concluded that *E. coli* and other Gram-negative bacteria have a higher tolerance to any synergistic effects of phenolic compounds.

(6) Banana peel extracts and their individual derived compounds used in this study are ineffective in reducing MS2 viral load, with no remarkable reduction being observed between the treated sample and the control. This implies that the banana peel extracts may not be suitable as a virucide, although testing at lower pH values and on other virus types is recommended.

(7) Under the experimental setup used in this study, there are detectable but limited amounts of hydrogen peroxide generated by the individual compounds (i.e. less than 100 μM) and not enough for significant reduction in bacterial growth to be observed. The inhibitory activity observed by EGCg towards *S. aureus* as noted in this study was not due to hydrogen peroxide generation only but by other mechanisms, most likely direct action of the molecule against the cells. Further research is needed to confirm these mechanisms.

(8) The extracts and individual extract compounds studied in this research show promise to serve as elements within a mixture of natural compounds to form disinfectants or cleaning products. However they are not suitable for use on their own due to the high concentrations needed and/or their limited effectiveness against a wide enough range of target organisms. Further research is recommended to observe the antimicrobial activity of these compounds when considered alongside other plant-derived biocide compounds (e.g. derived from other fruit peels or crop wastes) and in combination with surfactants.

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## Appendices

## Appendix A: Supplementary information for extraction procedure and compounds identification

### Appendix A1: Raw data for extraction procedures

Table A 1: Raw data for solute/solute ratio

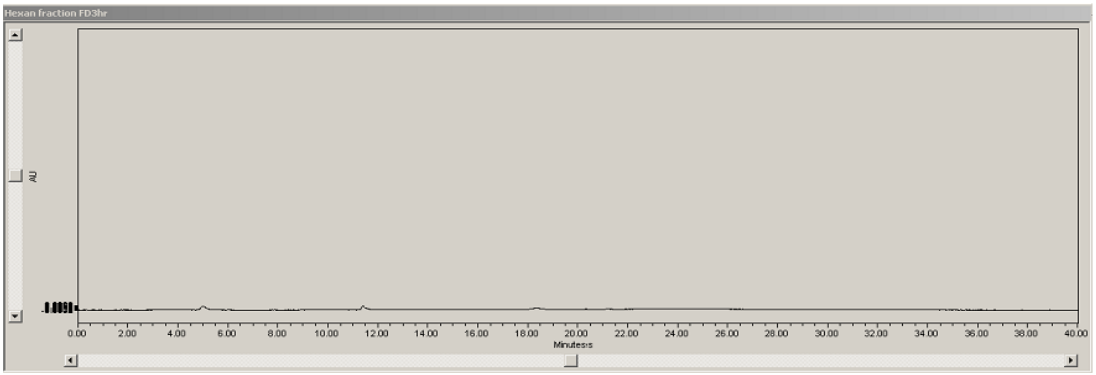
| solid/solute ratio (g/mL) | R1 | R2 | R3 | Mean Recovery (%) | STDEV |
|---|---|---|---|---|---|
| 0.02 | 29.00 | 21.27 | 23.29 | 24.52 | 4.01 |
| 0.04 | 35.46 | 28.97 | 34.54 | 32.99 | 3.51 |
| 0.06 | 25.93 | 37.19 | 33.42 | 32.18 | 5.73 |
| 0.08 | 21.63 | 20.51 | 27.68 | 23.27 | 3.86 |
| 0.1 | 15.80 | 19.67 | 18.29 | 17.92 | 1.96 |
| 0.12 | 18.77 | 10.13 | 12.70 | 13.86 | 4.44 |

Table A 2: Raw data for extraction yield at different maceration time

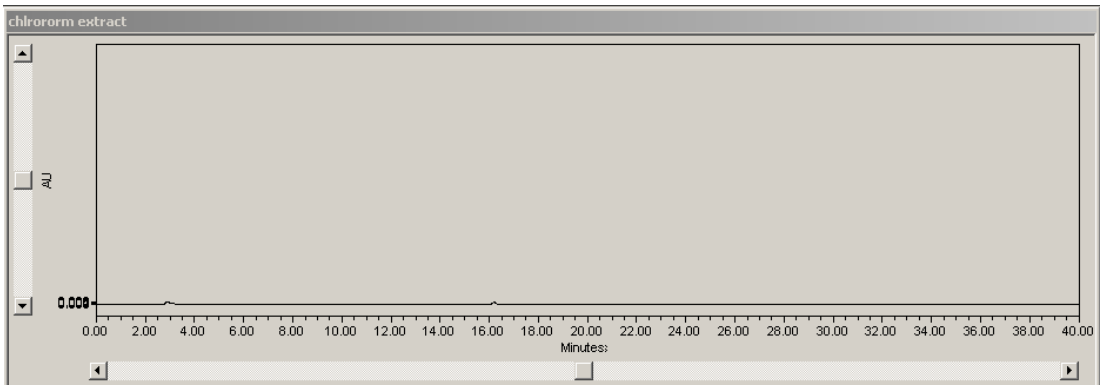
| Sample | FD | | | | | | OD | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Extraction time (hour) | 1 | 3 | 6 | 12 | 24 | 48 | 1 | 3 | 6 | 12 | 24 | 48 |
| R1 | 1.9 | 2.2 | 2.2 | 2.6 | 2.5 | 2.5 | 0.8 | 1.3 | 1.4 | 1.8 | 1.3 | 1.4 |
| R2 | 2.3 | 2.2 | 2.3 | 2.3 | 2.6 | 2.5 | 1.2 | 1.4 | 1.4 | 1.6 | 1.3 | 1.4 |
| R3 | 2.2 | 2.4 | 2.6 | 2.3 | 2.7 | 3.0 | 1.1 | 1.6 | 1.5 | 1.8 | 1.3 | 1.4 |
| Mean (g/10g DW) | 2.1 | 2.3 | 2.4 | 2.4 | 2.6 | 2.7 | 1.0 | 1.5 | 1.4 | 1.8 | 1.3 | 1.4 |
| Stdev | 0.2 | 0.1 | 0.2 | 0.2 | 0.1 | 0.3 | 0.2 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 |
| Yield (g/100 g DW) | 21.1 | 22.6 | 23.8 | 23.8 | 25.9 | 26.9 | 10.3 | 14.5 | 14.4 | 17.6 | 13.1 | 14.0 |

FD- Freeze-dried
OD- Oven-dried

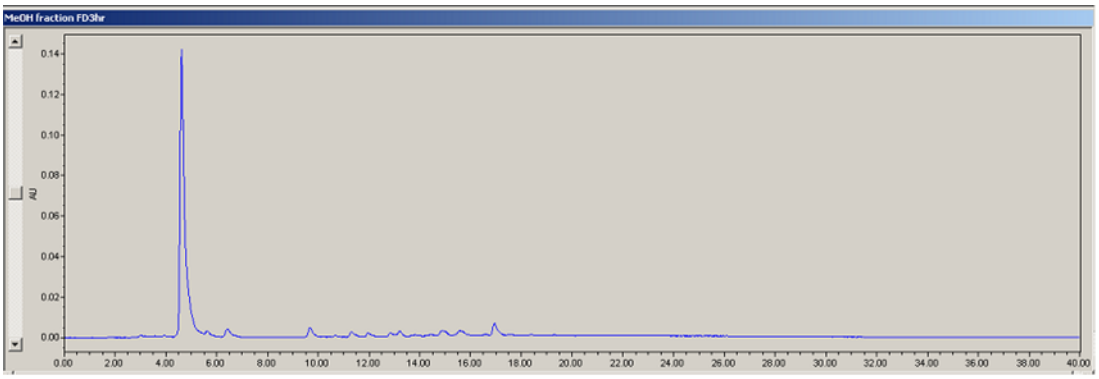
**Appendix A2: Fractionation of methanolic extracts with hexane and chloroform**




(a)



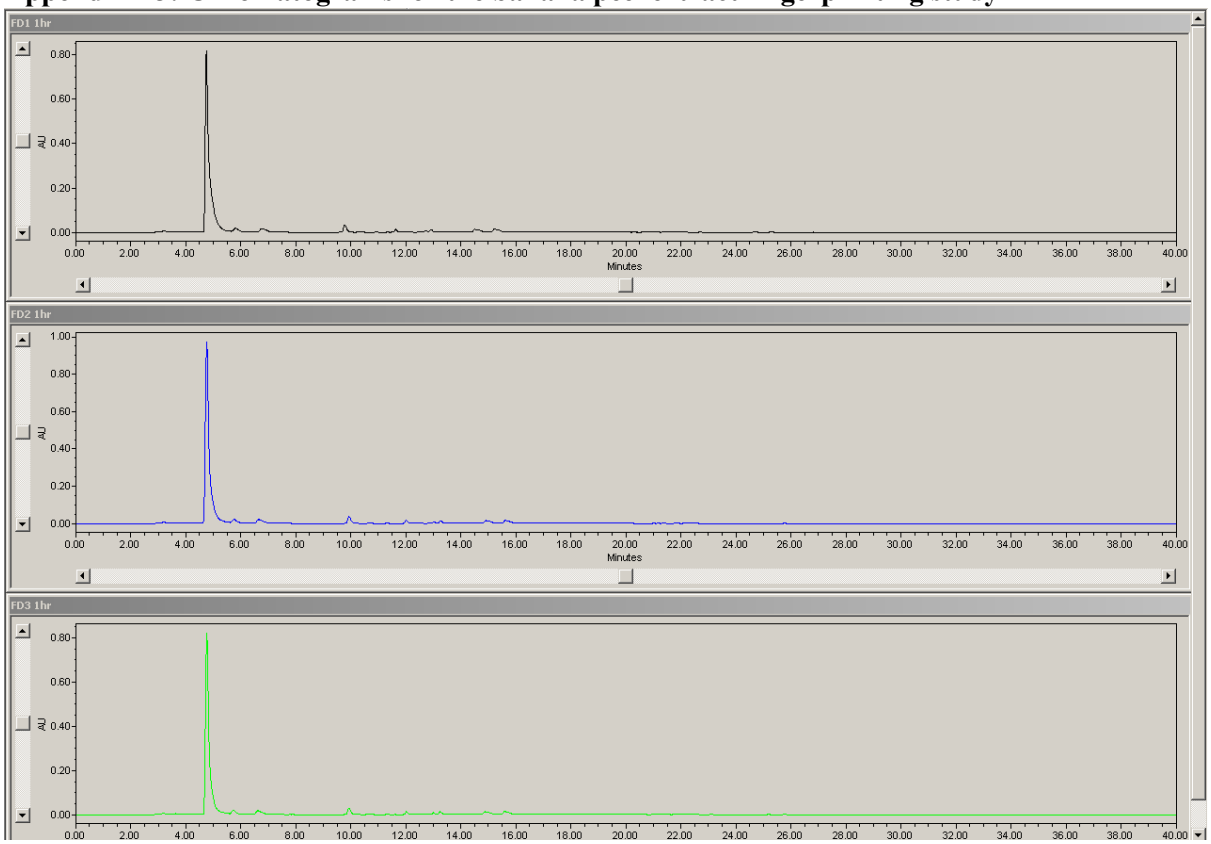

(b)



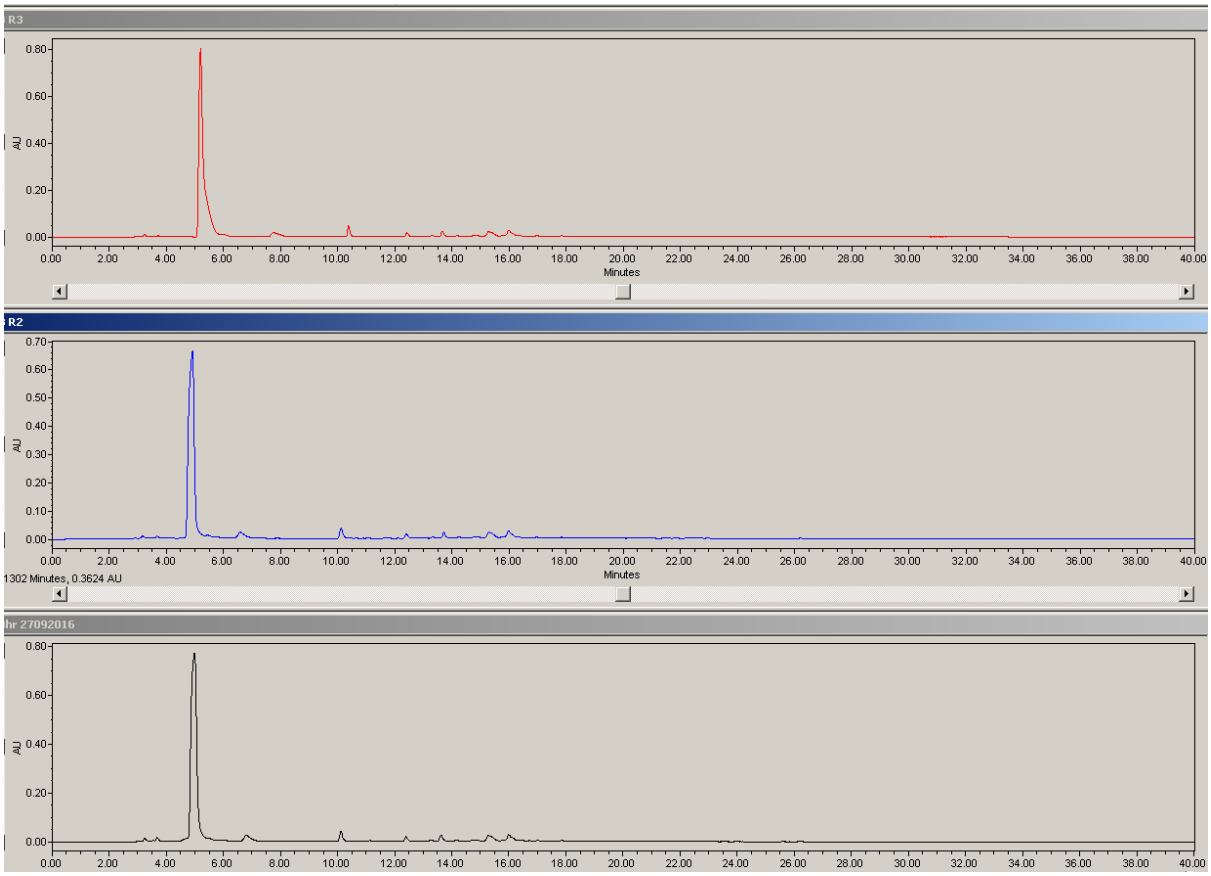

(c)

***Figure A 1:*** *Chromatogram of (a) hexane fraction (b) chloroform fraction and (c) methanolic fraction of freeze dried banana extracts.* As observed, no compounds can be detected in the hexane and chloroform extracts. The scale of y-axis for each of chromatograms are in the same range.

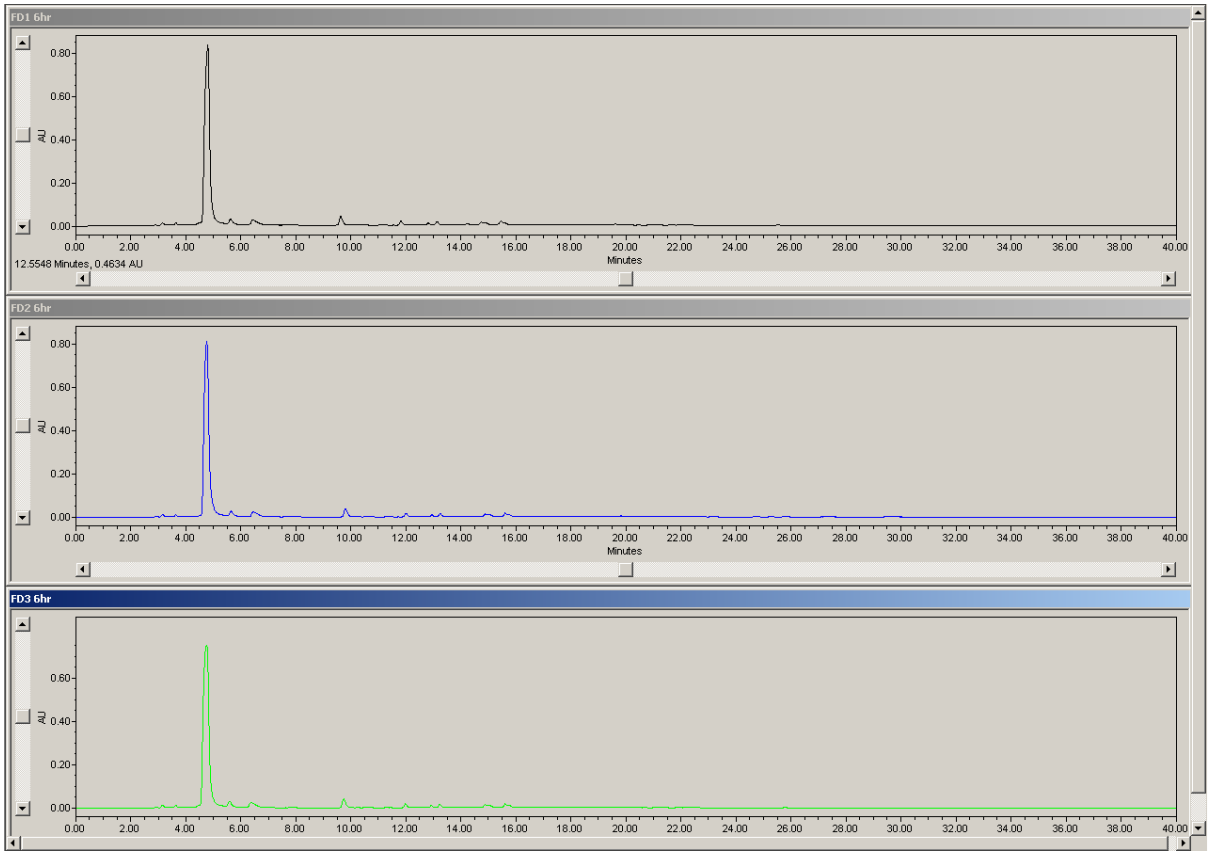
## Appendix A3: Chromatograms for the banana peel extract fingerprinting study



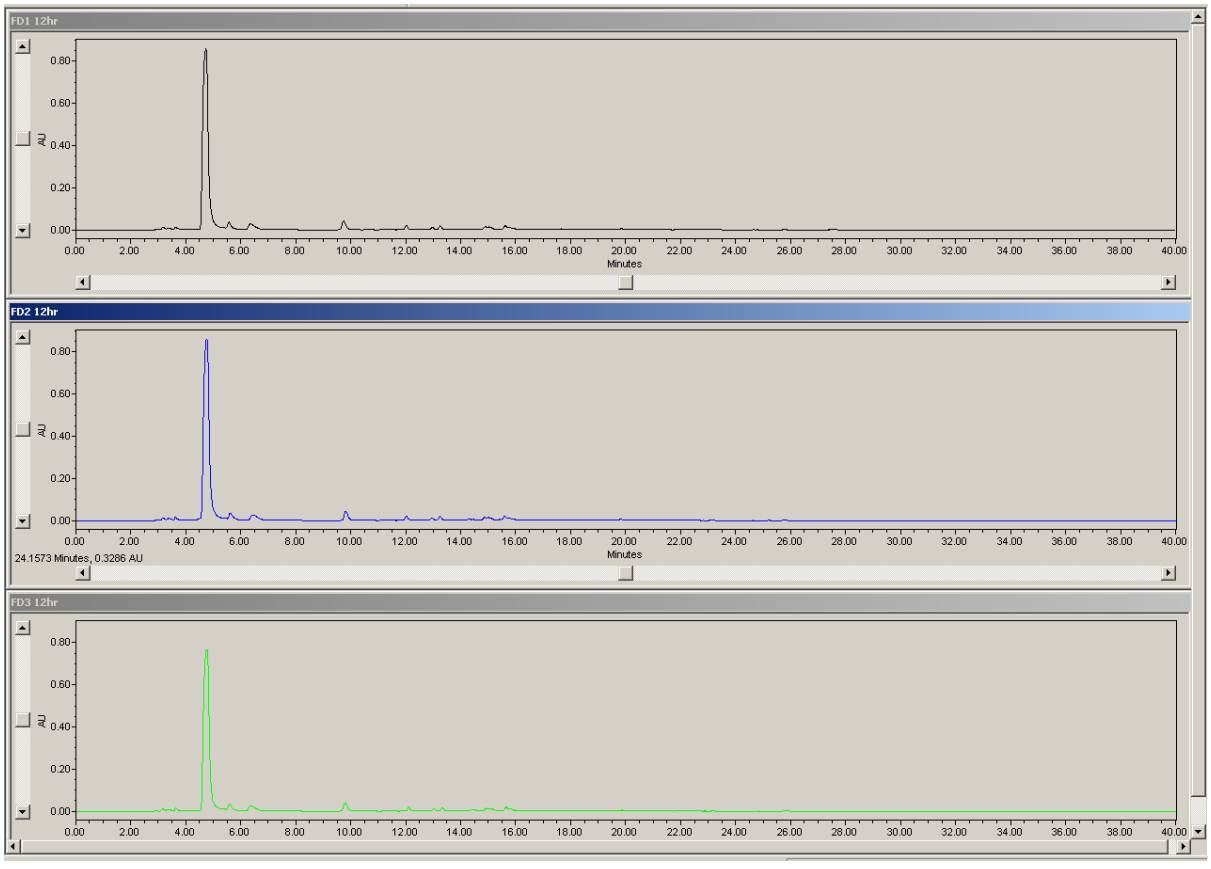

(a)



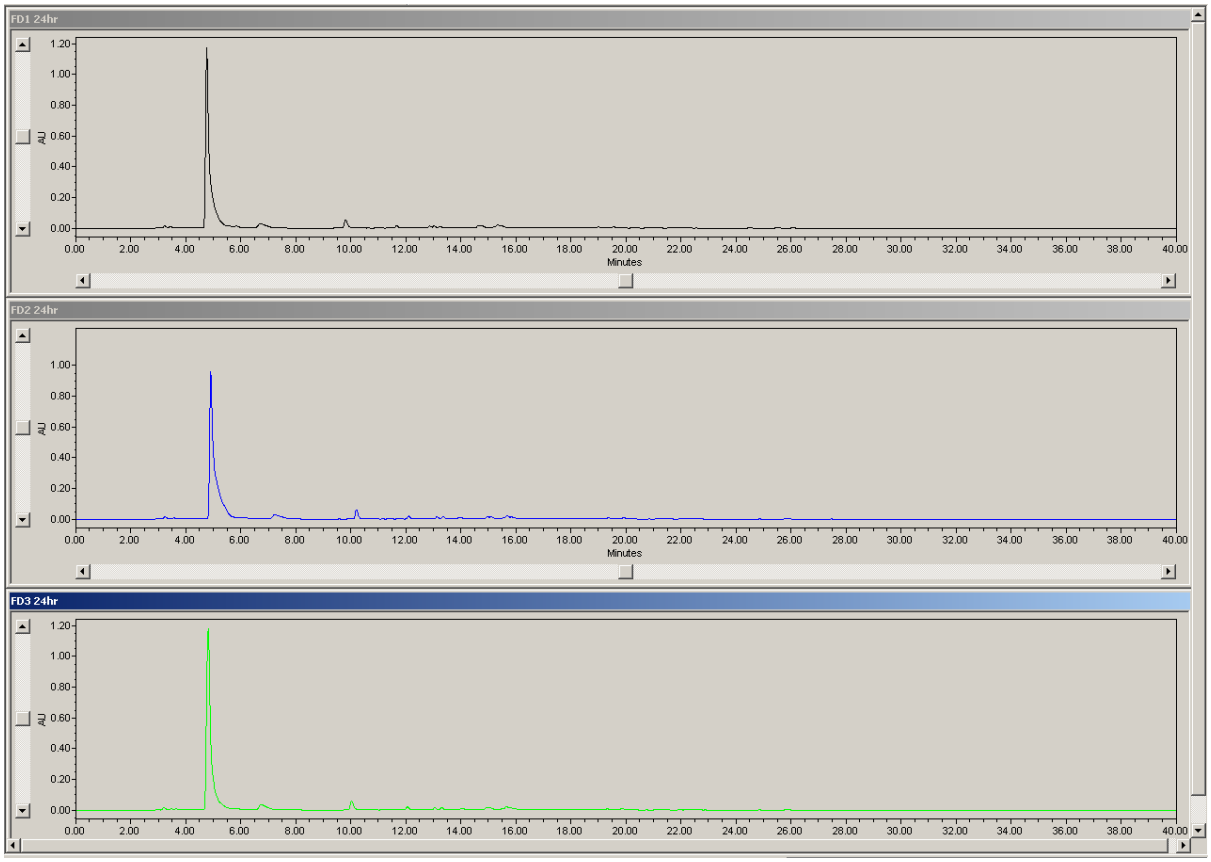

(b)


FD1 6hr
FD2 6hr
FD3 6hr
AU
Minutes
12.5548 Minutes, 0.4634 AU

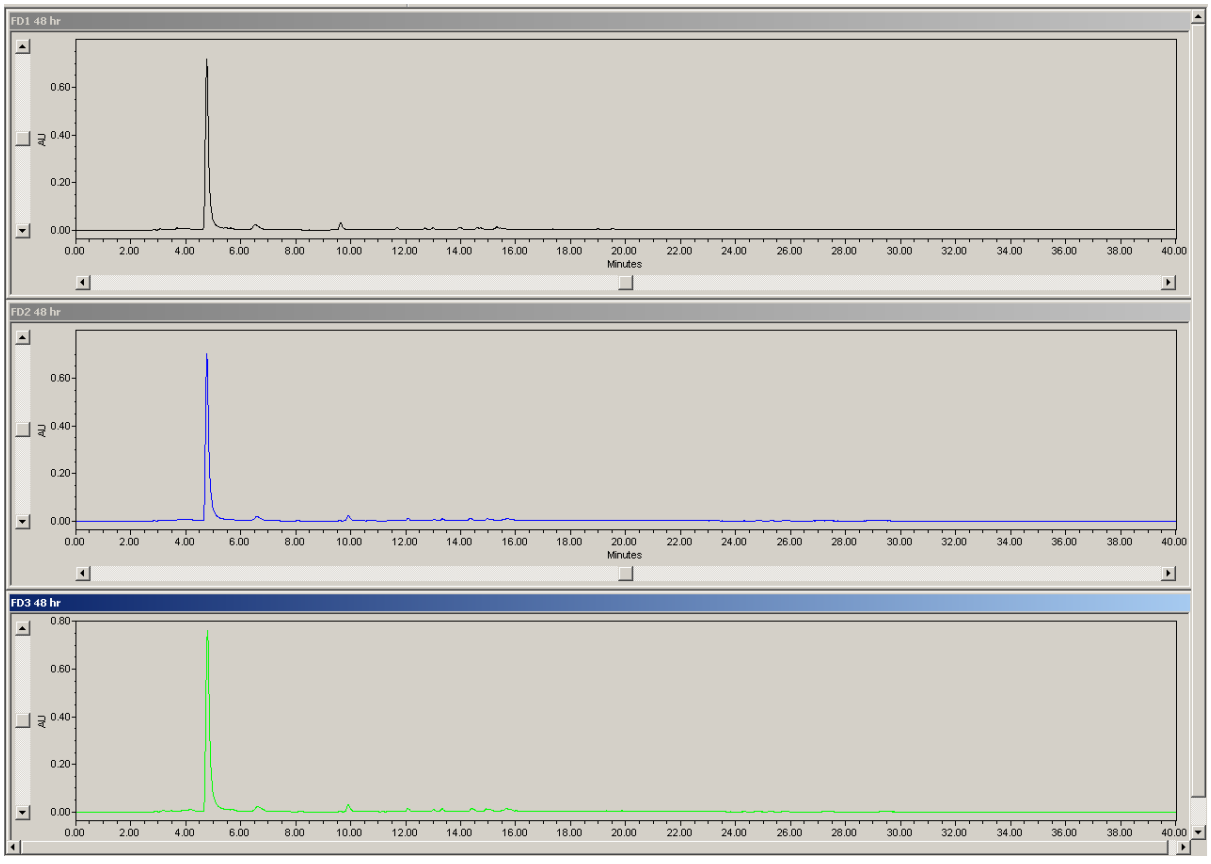
(c)


FD1 12hr
FD2 12hr
FD3 12hr
AU
Minutes
24.1573 Minutes, 0.3286 AU

(d)


FD1 24hr
FD2 24hr
FD3 24hr
AU
Minutes

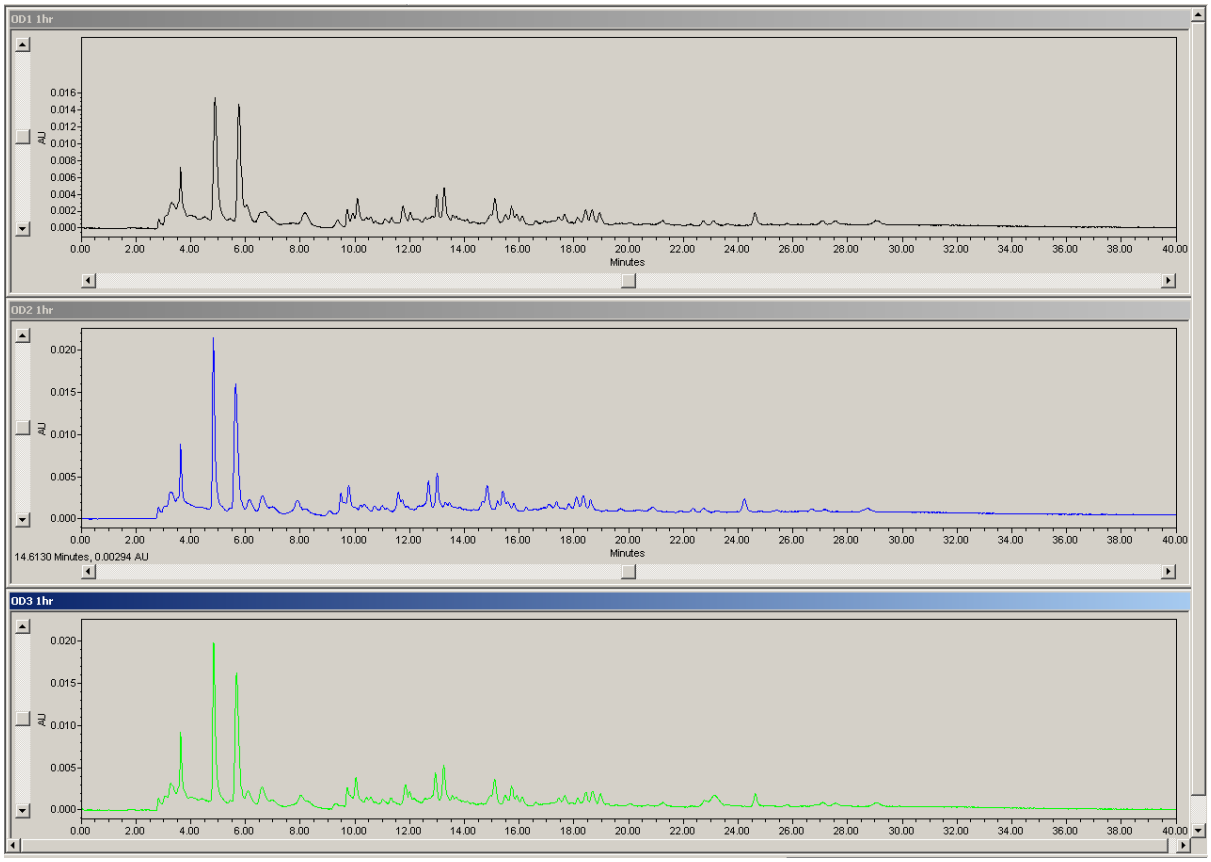
(e)


FD1 48 hr
FD2 48 hr
FD3 48 hr
AU
Minutes

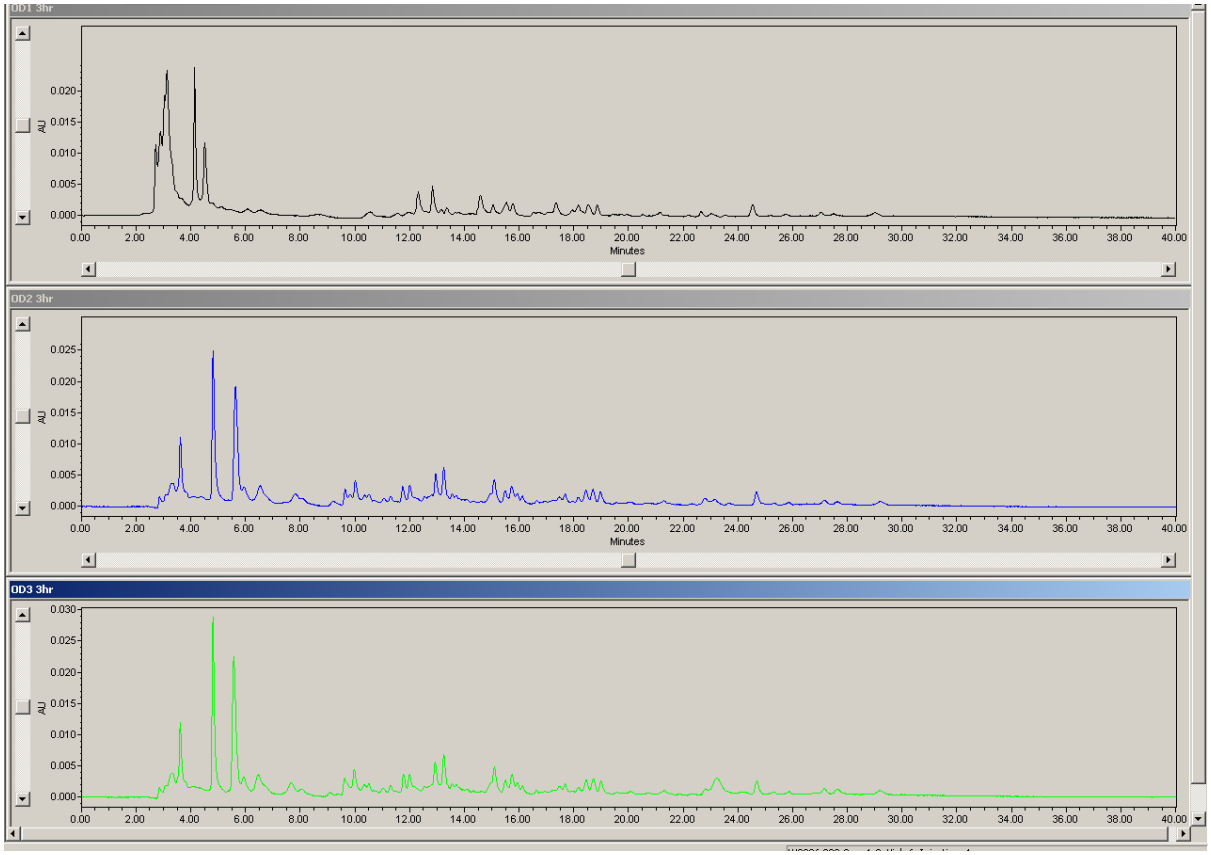
(f)

Figure A 2: fingerprinting study for Freeze-dried extracts at (a) 1hr, (b) 3hr, (c) 6hr, (d) 12hr, (e) 24 hr and (f) 48hr

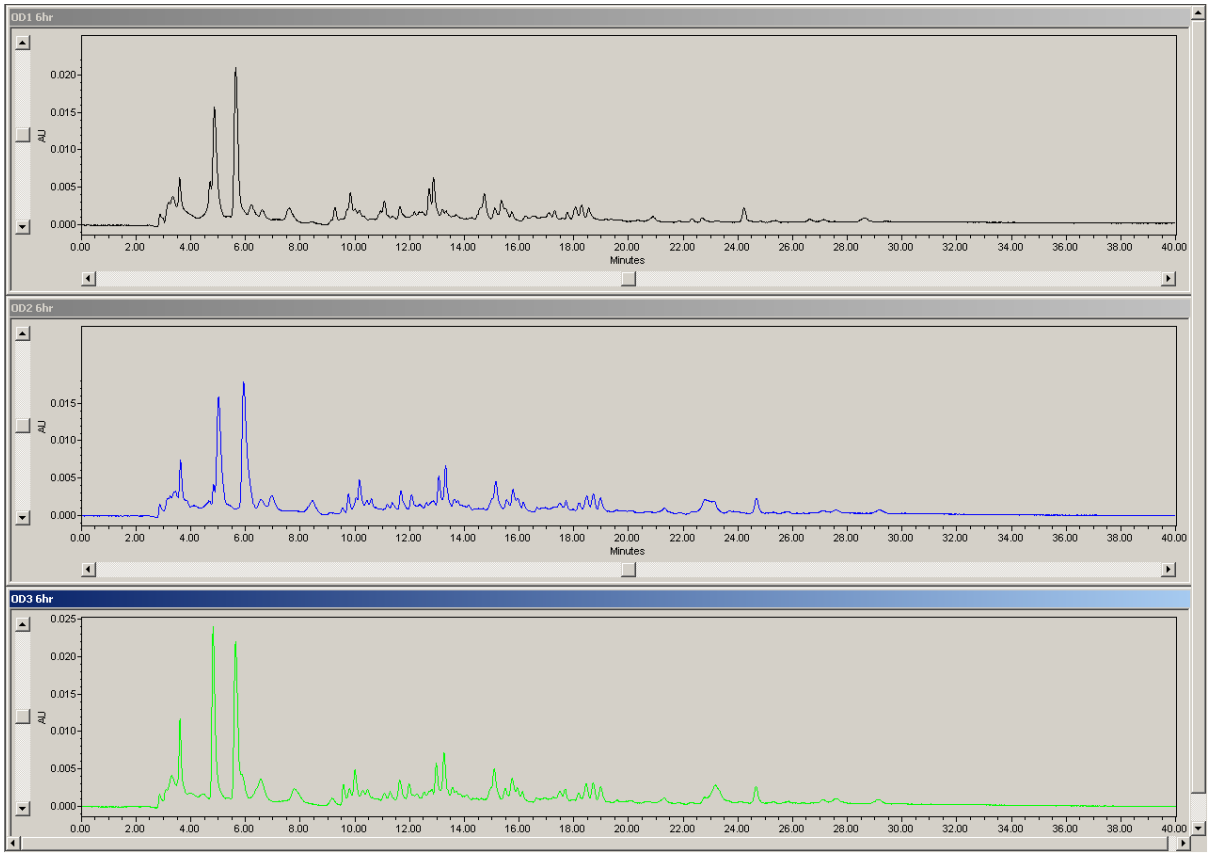
**all chromatograms are based on three independent samples.



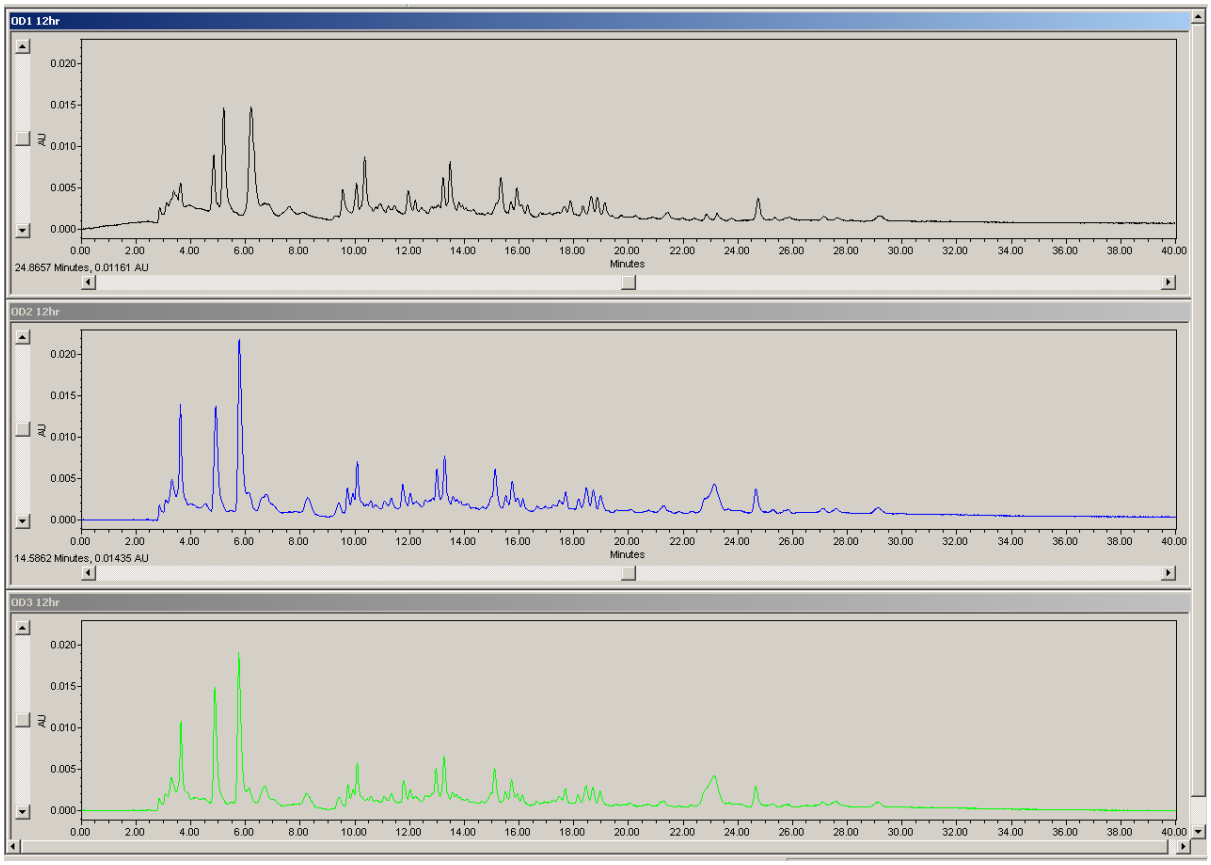

(a)


OD1 3hr
OD2 3hr
OD3 3hr
AU
Minutes

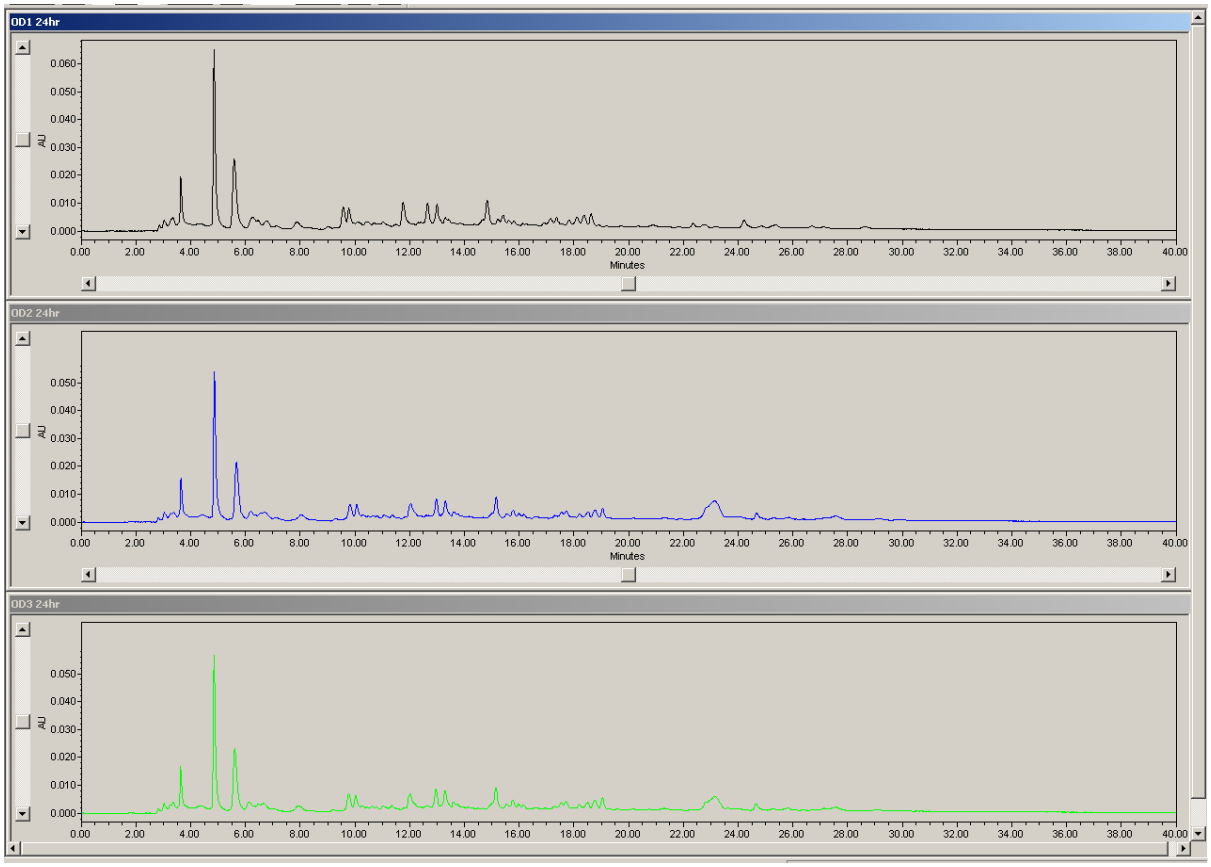
(b)


OD1 6hr
OD2 6hr
OD3 6hr
AU
Minutes

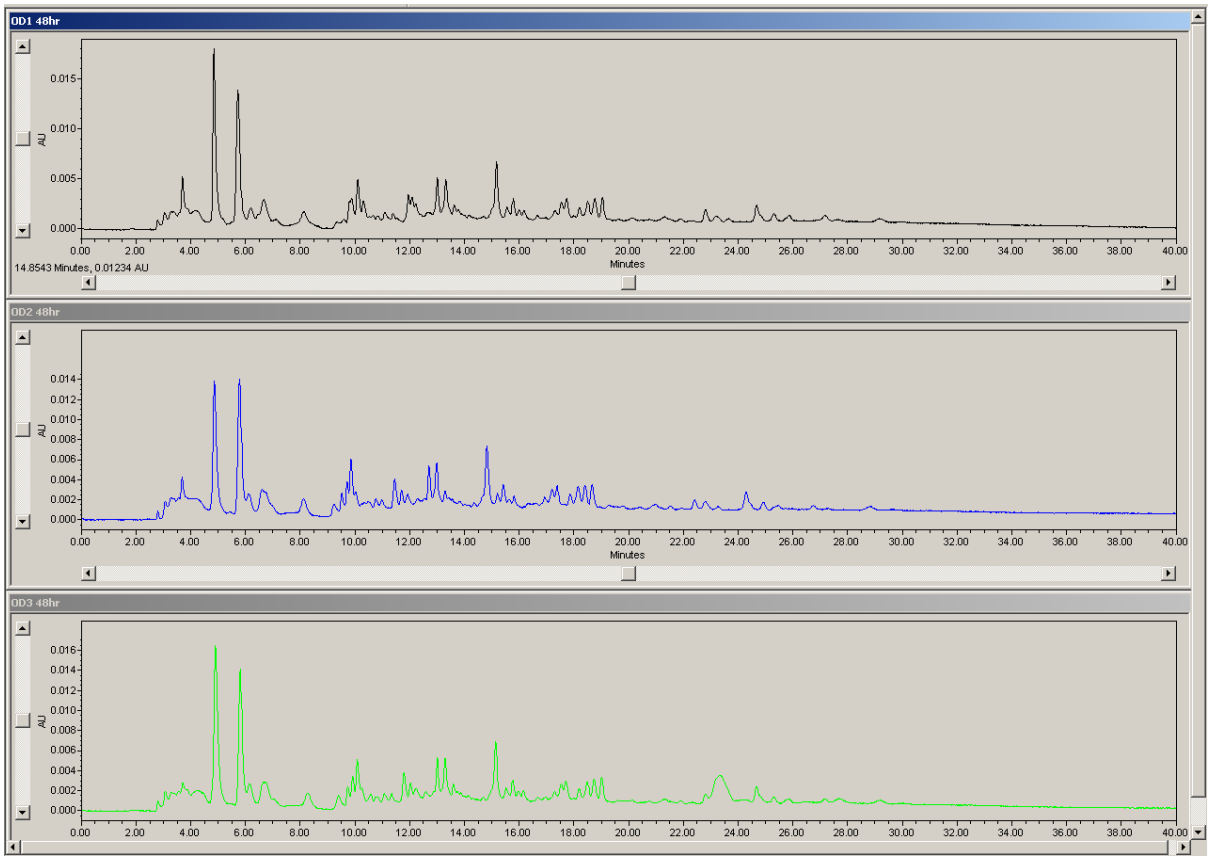
(c)


OD1 12hr
AU
Minutes
24.8657 Minutes, 0.01161 AU
OD2 12hr
AU
Minutes
14.5862 Minutes, 0.01435 AU
OD3 12hr
AU

(d)


OD1 24hr
AU
Minutes
OD2 24hr
AU
Minutes
OD3 24hr
AU

(e)

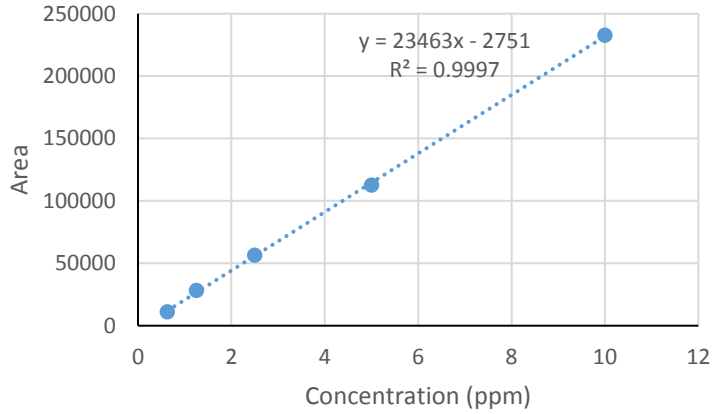



(f)

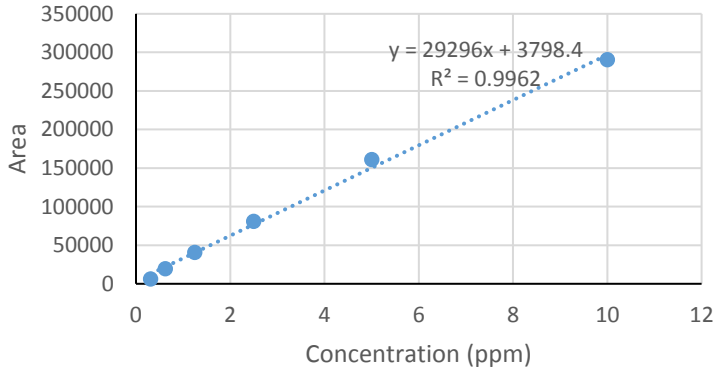
Figure A 3: Fingerprinting for oven-dried banana extracts at (a) 1hr, (b) 3hr, (c) 6hr, (d) 12hr, (e) 24 hr and (f) 48 hr

**all chromatograms are based on three independent samples.

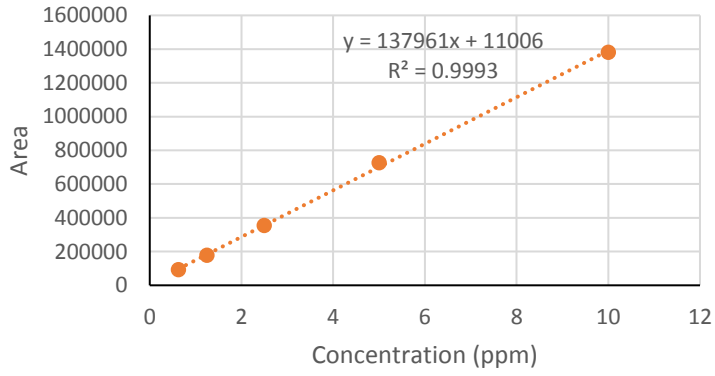
**Appendix A4: Supplementary data for HPLC procedures**




(a)

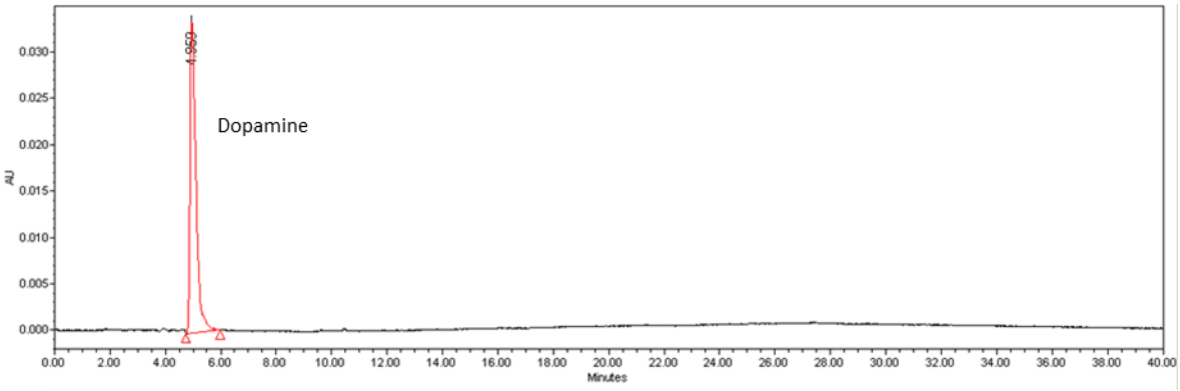



(b)

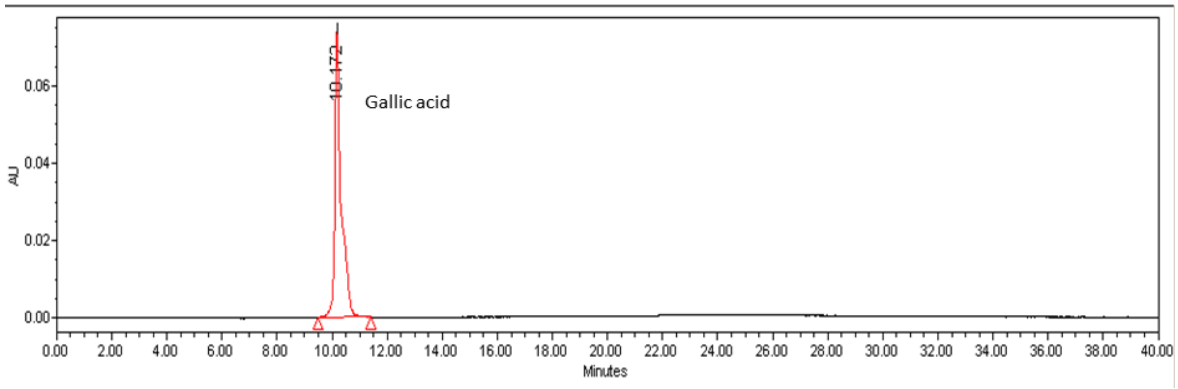



(c)

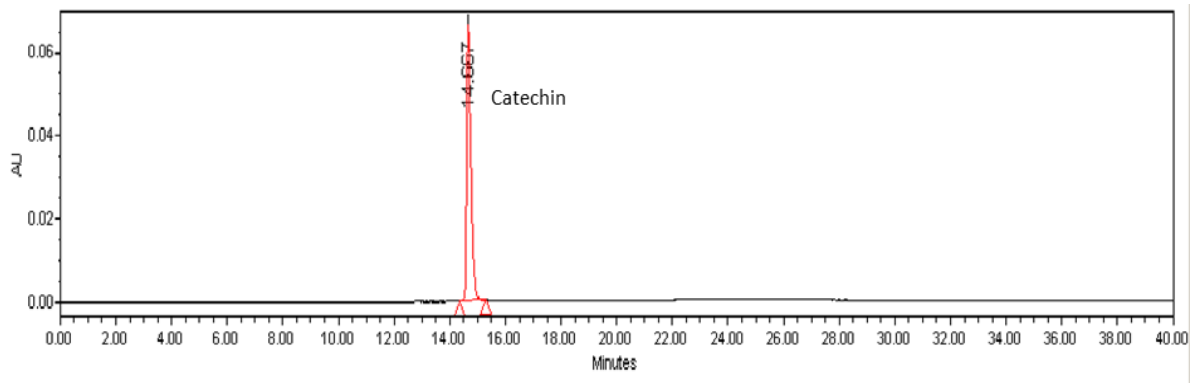
Figure A 4: Standard calibration curve for (a) catechin (b) epicatechin and (c) gallic acid



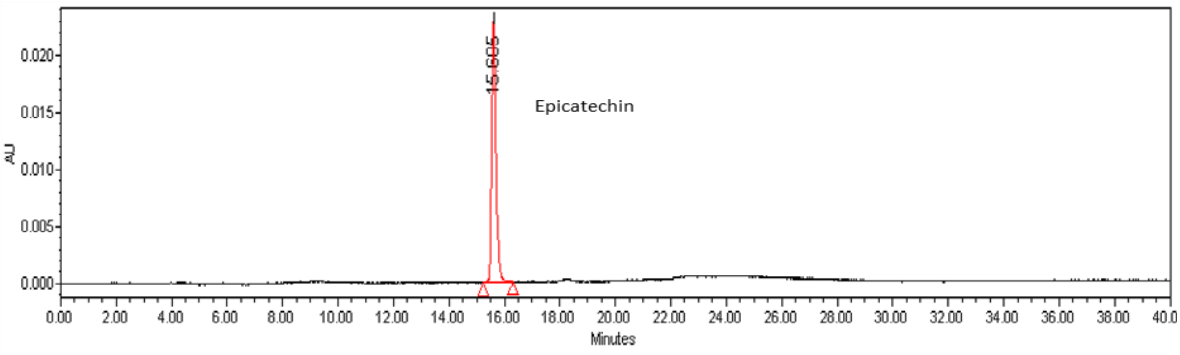

(a)




(b)




(c)




(d)

Figure A 5: Elution time for standard compounds: (a) dopamine, (b) gallic acid, (c) catechin and (d) epicatechin.

Table A 3: Composition of catechin in Freeze-dried banana extracts

| Extraction time (hr) | 1 | 3 | 6 | 12 | 24 | 48 |
|---|---|---|---|---|---|---|
| R1 | 422.55 | 454.17 | 281.6998 | 277.55 | 316.08 | 93.97 |
| R2 | 281.99 | 420.46 | 281.8362 | 245.47 | 228.92 | 86.30 |
| R3 | 290.71 | 396.46 | 364.3391 | 258.74 | 338.23 | 112.37 |
| Mean | 331.75 | 423.70 | 309.29 | 260.59 | 294.41 | 97.54 |
| Stdev | 78.75 | 28.99 | 47.67 | 16.12 | 57.79 | 13.40 |

Table A 4: Composition of epicatechin in Freeze-dried banana extracts

| Extraction time (hr) | 1 | 3 | 6 | 12 | 24 | 48 |
|---|---|---|---|---|---|---|
| R1 | 341.42 | 217.40 | 222.11 | 159.00 | 162.08 | 51.48 |
| R2 | 247.03 | 203.86 | 209.61 | 148.07 | 165.57 | 61.68 |
| R3 | 222.81 | 200.26 | 205.72 | 213.39 | 228.95 | 52.32 |
| Mean | 270.42 | 207.17 | 212.48 | 173.49 | 185.53 | 55.16 |
| Stdev | 62.67 | 9.04 | 8.57 | 34.99 | 37.64 | 5.67 |

Table A 5: Composition of gallic acid in Freeze-dried banana extracts

| | 1 | 3 | 6 | 12 | 24 | 48 |
|---|---|---|---|---|---|---|
| R1 | 55.59 | 62.14 | 68.72 | 36.62 | 46.81 | 10.38 |
| R2 | 56.36 | 46.37 | 66.65 | 31.38 | 44.83 | 9.93 |
| R2 | 52.00 | 42.57 | 68.77 | 38.01 | 32.02 | 11.10 |
| Mean | 54.65 | 50.36 | 68.05 | 35.34 | 41.22 | 10.47 |
| Stdev | 2.33 | 10.38 | 1.21 | 3.50 | 8.03 | 0.59 |

**Appendix B: Supplementary information for MIC study**

**Appendix B1: MIC experiment culture plate**

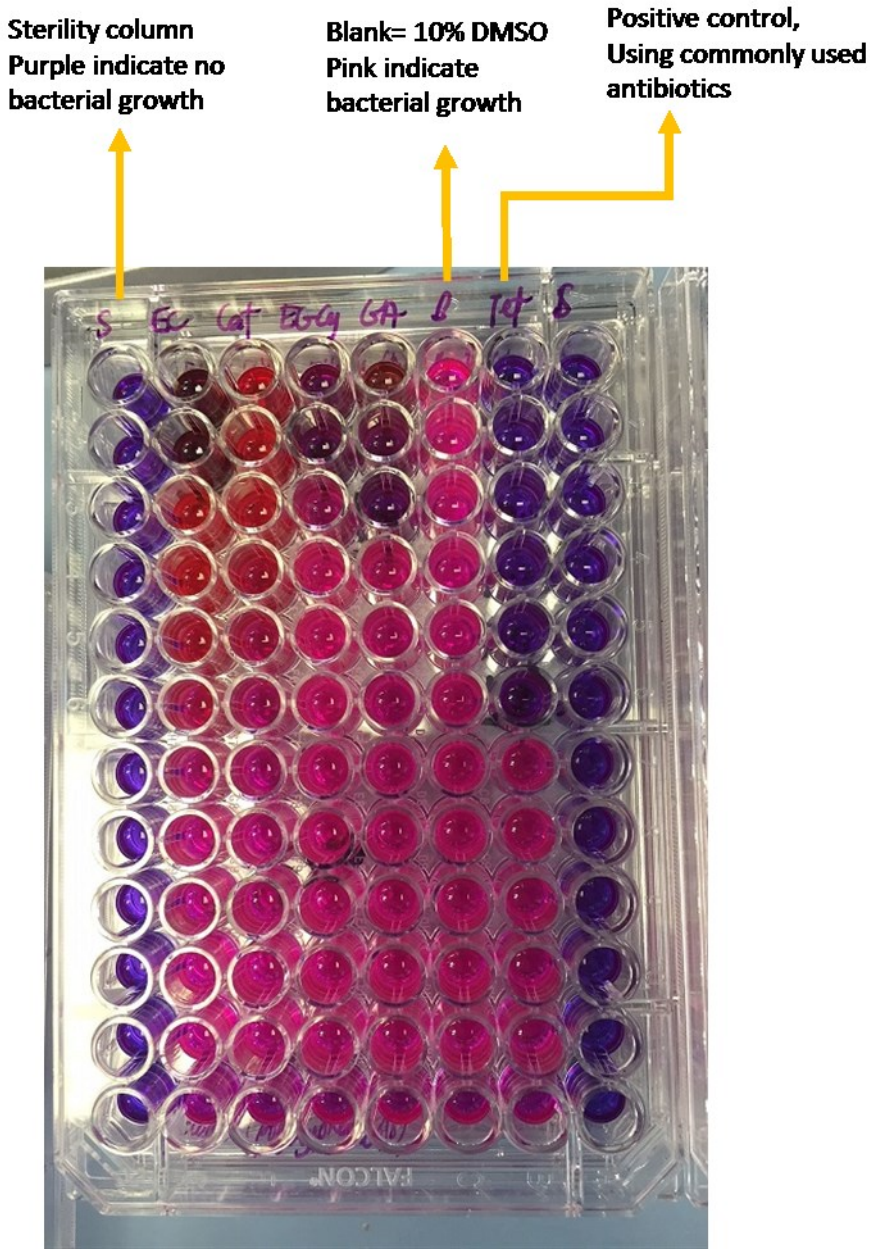



Figure B 1: Example of MIC culture plate after incubation

**Appendix B2: Raw data for MIC determination in the extracts**

Table B 1: MIC data for oven-dried banana extracts on *E. coli* and *S. aureus*

| Oven-dried | *E.coli* | | | | | *S.aureus* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Replicates | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV |
| 1hr | 12.50 | 12.50 | 12.50 | 12.50 | 0.00 | 6.25 | 6.25 | 12.50 | 8.33 | 3.61 |
| 3hr | 12.50 | 25.00 | 12.50 | 16.67 | 7.22 | 12.50 | 12.50 | 12.50 | 12.50 | 0.00 |
| 6 hr | 25.00 | 25.00 | 25.00 | 25.00 | 0.00 | 6.25 | 6.25 | 6.25 | 6.25 | 0.00 |
| 12hr | 12.50 | 12.50 | 12.50 | 12.50 | 0.00 | 12.50 | 12.50 | 12.50 | 12.50 | 0.00 |
| 24hr | 25.00 | 25.00 | 25.00 | 25.00 | 0.00 | 12.50 | 12.50 | 12.50 | 12.50 | 0.00 |
| 48hr | 25.00 | 25.00 | 25.00 | 25.00 | 0.00 | 12.50 | 12.50 | 12.50 | 12.50 | 0.00 |

**all data in mg/ml

Table B 2: MIC data for freeze-dried banana extracts on *E. coli* and *S. aureus*

| Freeze-dried | *E.coli* | | | | | *S.aureus* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Replicates | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV |
| 1 hr | 2.50 | 1.25 | 1.25 | 1.67 | 0.72 | 0.63 | 0.63 | 0.63 | 0.63 | 0.00 |
| 3hr | 1.25 | 1.25 | 1.25 | 1.25 | 0.00 | 0.63 | 0.63 | 0.63 | 0.63 | 0.00 |
| 6hr | 2.50 | 2.50 | 5.00 | 3.33 | 1.44 | 0.63 | 0.63 | 0.63 | 0.63 | 0.00 |
| 12hr | 2.50 | 2.50 | 2.50 | 2.50 | 0.00 | 1.25 | 1.25 | 2.50 | 1.67 | 0.72 |
| 24hr | 5.00 | 5.00 | 5.00 | 5.00 | 0.00 | 2.50 | 2.50 | 2.50 | 2.50 | 0.00 |
| 48hr | 5.00 | 5.00 | 10.00 | 6.67 | 2.89 | 5.00 | 2.50 | 5.00 | 4.17 | 1.44 |

**all data in mg/ml

**Appendix B3: Quality control using commercial antibiotics for MIC**

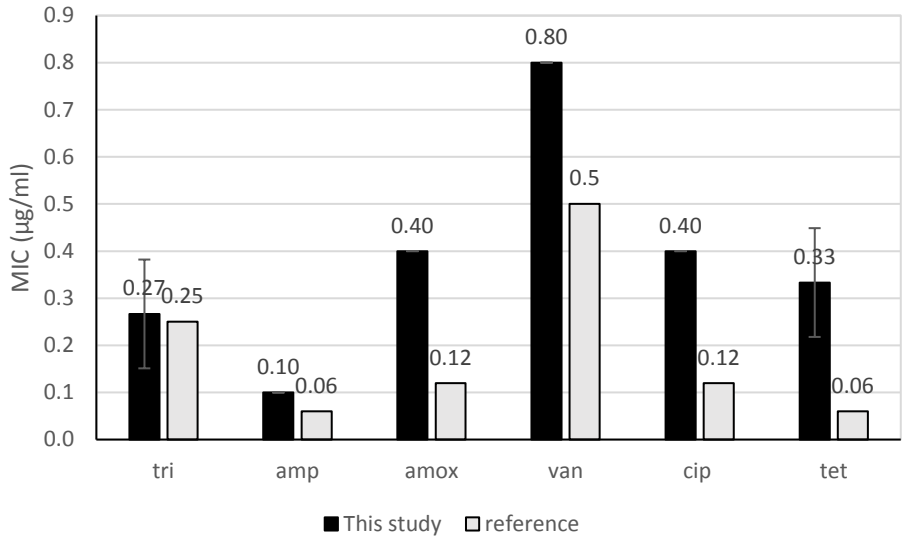



Figure B 2: Sensitivity of *S.aureus* NCTC 6571 to commercial antibiotics, as comparison to data published by Andrews (2001). Tri: trimethoprim, amp: ampicillin, amox: amoxicillin, van: vancomycin, cip: ciprofloxacin and tet: tetracycline.

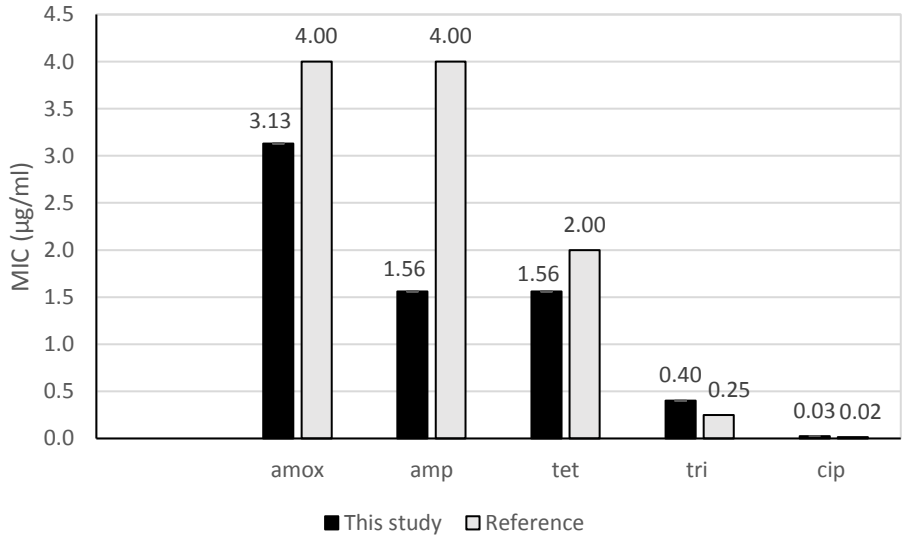



Figure B 3: Sensitivity of *E.coli* NCTC 12241 to commercial antibiotics, as comparison to data published by Andrews (2001). Amox: amoxicillin, amp: ampicillin, tet: tetracycline, tri: trimethoprim, and cip:ciprofloxacin. The MIC for vancomycin to *E. coli* NCTC12241 was not defined by Andrews (2001), therefore it was not tested with vancomycin.

**Appendix C Supplementary information for disinfection study**

**Appendix C1: Quality control for *S. aureus* and *E. coli***

Table C 1: Enumeration of *S. aureus* in PBS

| contact time (min) | Replicate | R1 | R2 | R3 |
|---|---|---|---|---|
| 0 | No. of colony | 136 | 118 | 141 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 13600000 | 11800000 | 14100000 |
| | average cfu/ml | 13166666.67 | | |
| | Log cfu/ml | 7.1 | | |
| 15 | No. of colony | 136 | 118 | 141 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 13600000 | 11800000 | 14100000 |
| | average cfu/ml | 13166666.67 | | |
| | Log cfu/ml | 7.1 | | |
| 30 | No. of colony | 131 | 135 | 152 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 13100000 | 13500000 | 15200000 |
| | average cfu/ml | 13933333.33 | | |
| | Log cfu/ml | 7.1 | | |
| 60 | No. of colony | 150 | 157 | 196 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 15000000 | 15700000 | 19600000 |
| | average cfu/ml | 16766666.67 | | |
| | Log cfu/ml | 7.2 | | |
| 120 | No. of colony | 132 | 170 | 163 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 13200000 | 17000000 | 16300000 |
| | average cfu/ml | 15500000 | | |
| | Log cfu/ml | 7.2 | | |
| 240 | No. of colony | 141 | 160 | 157 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 14100000 | 16000000 | 15700000 |
| | average cfu/ml | 15266666.67 | | |
| | Log cfu/ml | 7.2 | | |
| 360 | No. of colony | 140 | 152 | 150 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 14000000 | 15200000 | 15000000 |
| | average cfu/ml | 14733333.33 | | |
| | Log cfu/ml | 7.2 | | |

Table C 2: Enumeration of *E. coli* in PBS

| contact time (min) | Replicate | R1 | R2 | R3 |
|---|---|---|---|---|
| | No. of colony | 130 | 122 | 143 |
| | dilution | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 13000000 | 12200000 | 14300000 |
| | average cfu/ml | 13166666.7 | | |
| 0 | log cfu/ml | 7.1 | | |
| | No. of colony | 122 | 117 | 129 |
| | dilution | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 12200000 | 11700000 | 12900000 |
| | average cfu/ml | 12266666.7 | | |
| 15 | log cfu/ml | 7.1 | | |
| | No. of colony | 128 | 127 | 108 |
| | dilution | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 12800000 | 12700000 | 10800000 |
| | average cfu/ml | 12100000.0 | | |
| 30 | log cfu/ml | 7.1 | | |
| | No. of colony | 122 | 141 | 119 |
| | dilution | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 12200000 | 14100000 | 11900000 |
| | average cfu/ml | 12733333.3 | | |
| 60 | log cfu/ml | 7.1 | | |
| | No. of colony | 123 | 120 | 119 |
| | dilution | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 12300000 | 12000000 | 11900000 |
| | average cfu/ml | 12066666.7 | | |
| 120 | log cfu/ml | 7.1 | | |
| | No. of colony | 119 | 126 | 124 |
| | dilution | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 11900000 | 12600000 | 12400000 |
| | average cfu/ml | 12300000.0 | | |
| 240 | log cfu/ml | 7.1 | | |
| | No. of colony | 122 | 123 | 123 |
| | dilution | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 12200000 | 12300000 | 12300000 |
| | average cfu/ml | 12266666.7 | | |
| 360 | log cfu/ml | 7.1 | | |

**Appendix C2: Raw data for *S.aureus* disinfection**

Table C 3: Effect of banana extracts on *S. aureus* growth

| | | FD3hr 0.5 MIC | | | | FD3hr 1MIC | | | | FD3hr 2MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| contact time (min) | sample | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | mean colonies | 62.0 | 63.0 | 65.0 | 62.3 | 101.7 | 104.3 | 108.0 | 113.0 | 107.7 | 110.3 | 118.7 | 109.7 |
| | stdev | 8.5 | 7.8 | 16.7 | 23.6 | 20.0 | 22.4 | 14.4 | 18.0 | 26.6 | 24.0 | 16.5 | 30.6 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | CFU | 620000.0 | 630000.0 | 650000.0 | 623333.3 | 1016666.7 | 1043333.3 | 1080000.0 | 1130000.0 | 1076666.7 | 1103333.3 | 1186666.7 | 1096666.7 |
| | $Log_{10}(N_o/N)$ | 0.0 | 0.0 | 0.0 | | 0.0 | 0.0 | 0.0 | | 0.0 | 0.0 | 0.0 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | average | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 60.0 | 50.0 | 40.0 | 53.7 | 83.3 | 78.0 | 64.0 | 112.7 | 80.7 | 66.3 | 83.3 | 112.3 |
| | stdev | 14.7 | 8.7 | 8.7 | 7.4 | 21.6 | 10.1 | 3.6 | 6.4 | 3.5 | 15.7 | 5.1 | 10.8 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | CFU | 600000.0 | 500000.0 | 400000.0 | 536666.7 | 833333.3 | 780000.0 | 640000.0 | 1126666.7 | 806666.7 | 663333.3 | 833333.3 | 1123333.3 |
| | $Log_{10}(N_o/N)$ | 0.0 | 0.1 | 0.2 | | 0.1 | 0.1 | 0.2 | | 0.1 | 0.2 | 0.2 | |
| | mean | | | 0.1 | | | | 0.1 | | | | 0.2 | |
| 30 | average | | | 0.1 | | | | 0.1 | | | | 0.0 | |
| | mean colonies | 43.0 | 51.3 | 52.3 | 54.0 | 74.0 | 75.0 | 66.7 | 96.0 | 89.0 | 85.0 | 77.3 | 110.0 |
| | stdev | 7.5 | 18.1 | 12.4 | 1.0 | 9.5 | 12.5 | 5.9 | 12.8 | 16.1 | 13.9 | 19.7 | 19.7 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | CFU | 430000.0 | 513333.3 | 523333.3 | 540000.0 | 740000.0 | 750000.0 | 666666.7 | 960000.0 | 890000.0 | 850000.0 | 773333.3 | 1100000.0 |
| | $Log_{10}(N_o/N)$ | 0.2 | 0.1 | 0.1 | | 0.1 | 0.1 | 0.2 | | 0.1 | 0.1 | 0.2 | |
| | mean | | | 0.1 | | | | 0.2 | | | | 0.1 | |
| 60 | average | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| | mean colonies | 50.3 | 37.7 | 35.0 | 55.3 | 52.0 | 79.7 | 80.3 | 101.0 | 72.3 | 61.3 | 75.7 | 101.0 |
| | stdev | 15.0 | 1.5 | 5.6 | 2.5 | 8.0 | 5.9 | 7.6 | 32.2 | 3.1 | 3.2 | 6.5 | 14.9 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | CFU | 503333.3 | 376666.7 | 350000.0 | 553333.3 | 520000.0 | 796666.7 | 803333.3 | 1010000.0 | 723333.3 | 613333.3 | 756666.7 | 1010000.0 |
| | $Log_{10}(N_o/N)$ | 0.1 | 0.2 | 0.3 | | 0.3 | 0.1 | 0.1 | | 0.2 | 0.3 | 0.2 | |
| | mean | | | 0.2 | | | | 0.2 | | | | 0.2 | |
| 120 | average | | | 0.1 | | | | 0.1 | | | | 0.0 | |
| | mean colonies | 47.0 | 42.7 | 48.7 | 51.3 | 57.0 | 61.7 | 82.7 | 107.0 | 47.3 | 46.3 | 52.0 | 103.7 |
| | stdev | 9.2 | 9.0 | 8.1 | 2.3 | 5.3 | 8.0 | 27.1 | 16.0 | 11.7 | 1.5 | 7.2 | 3.2 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | CFU | 470000.0 | 426666.7 | 486666.7 | 513333.3 | 570000.0 | 616666.7 | 826666.7 | 1070000.0 | 473333.3 | 463333.3 | 520000.0 | 1036666.7 |
| | $Log_{10}(N_o/N)$ | 0.1 | 0.2 | 0.1 | | 0.3 | 0.2 | 0.1 | | 0.4 | 0.4 | 0.4 | |
| | mean | | | 0.1 | | | | 0.2 | | | | 0.4 | |
| 240 | average | | | 0.0 | | | | 0.1 | | | | 0.0 | |
| | mean colonies | 62.3 | 45.7 | 36.7 | 53.7 | 25.0 | 46.7 | 56.7 | 110.7 | 27.0 | 29.7 | 44.7 | 106.0 |
| | stdev | 29.0 | 13.7 | 24.5 | 4.0 | 15.4 | 6.5 | 10.7 | 11.0 | 3.6 | 4.5 | 8.4 | 1.7 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | CFU | 623333.3 | 456666.7 | 366666.7 | 536666.7 | 250000.0 | 466666.7 | 566666.7 | 1106666.7 | 270000.0 | 296666.7 | 446666.7 | 1060000.0 |
| | $Log_{10}(N_o/N)$ | 0.0 | 0.1 | 0.2 | | 0.6 | 0.3 | 0.3 | | 0.6 | 0.6 | 0.4 | |
| | mean | | | 0.1 | | | | 0.4 | | | | 0.5 | |
| 360 | average | | | 0.1 | | | | 0.2 | | | | 0.1 | |

Table C 4: Effect of Epigallocatechin gallate on *S. aureus* growth

| contact time (min) | samples | EGCg 1xMIC | | | | EGCg 2MIC | | | | EGCg 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | mean colonies | 24.33 | 23.67 | 24.67 | 25.00 | 216.33 | 201.33 | 203.00 | 207.33 | 199.67 | 199.00 | 197.33 | 208.33 |
| | stdev | 6.03 | 8.33 | 4.62 | 7.00 | 60.53 | 30.35 | 8.19 | 11.02 | 28.50 | 23.81 | 17.67 | 34.96 |
| | dilution factor | 10^-6 | 10^-6 | 10^-6 | 10^-6 | 10^-6 | 10^-6 | 10^-6 | 10^-6 | 10^-6 | 10^-6 | 10^-6 | 10^-6 |
| | CFU | 2433333.33 | 2366666.67 | 2466666.67 | 2500000.00 | 21633333.33 | 20133333.33 | 20300000.00 | 20733333.33 | 19966666.67 | 19900000.00 | 19733333.33 | 20833333.33 |
| | $Log_{10}(N_o/N)$ | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | average | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 69.33 | 75.67 | 69.00 | 37.67 | 55.67 | 106.33 | 153.00 | 186.00 | 169.00 | 159.33 | 167.00 | 154.67 |
| | stdev | 22.74 | 14.36 | 5.00 | 3.06 | 7.37 | 8.74 | 15.72 | 12.12 | 23.64 | 27.43 | 4.58 | 48.39 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-6 | 10^-5 | 10^-5 | 10^-5 | 10^-6 | 10^-5 | 10^-5 | 10^-5 | 10^-6 |
| | CFU | 693333.33 | 756666.67 | 690000.00 | 3766666.67 | 556666.67 | 1063333.33 | 1530000.00 | 18600000.00 | 1690000.00 | 1593333.33 | 1670000.00 | 15466666.67 |
| | $Log_{10}(N_o/N)$ | 0.55 | 0.50 | 0.55 | | 1.59 | 1.28 | 1.12 | 0.05 | 1.07 | 1.10 | 1.07 | 0.13 |
| | mean | | | 0.5 | | | | 1.3 | | | | 1.1 | |
| 30 | average | | | 0.0 | | | | 0.2 | | | | 0.0 | |
| | mean colonies | 52.00 | 43.67 | 67.67 | 38.33 | 46.33 | 82.67 | 60.67 | 183.33 | 83.00 | 102.67 | 105.33 | 183.00 |
| | stdev | 14.18 | 15.31 | 8.96 | 5.51 | 8.02 | 11.55 | 8.50 | 15.28 | 9.17 | 15.70 | 4.73 | 22.61 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-6 | 10^-4 | 10^-4 | 10^-4 | 10^-6 | 10^-4 | 10^-4 | 10^-4 | 10^-6 |
| | CFU | 520000.00 | 436666.67 | 676666.67 | 3833333.33 | 46333.33 | 82666.67 | 606666.67 | 18333333.33 | 83000.00 | 102666.67 | 105333.33 | 18300000.00 |
| | $Log_{10}(N_o/N)$ | 0.67 | 0.73 | 0.56 | | 2.67 | 2.39 | 1.52 | 0.05 | 2.38 | 2.29 | 2.27 | 0.06 |
| | mean | | | 0.7 | | | | 2.2 | | | | 2.3 | |
| 60 | average | | | 0.1 | | | | 0.6 | | | | 0.1 | |
| | mean colonies | 90.33 | 123.33 | 110.00 | 31.00 | 14.00 | 15.00 | 37.33 | 165.00 | 62.00 | 105.67 | 87.33 | 142.00 |
| | stdev | 5.69 | 32.56 | 2.65 | 12.29 | 3.46 | 1.00 | 6.11 | 15.00 | 11.00 | 9.61 | 7.37 | 15.10 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-6 | 10^-4 | 10^-4 | 10^-4 | 10^-6 | 10^-3 | 10^-3 | 10^-3 | 10^-6 |
| | CFU | 90333.33 | 123333.33 | 110000.00 | 3100000.00 | 14000.00 | 15000.00 | 37333.33 | 16500000.00 | 6200.00 | 10566.67 | 8733.33 | 14200000.00 |
| | $Log_{10}(N_o/N)$ | 1.43 | 1.28 | 1.35 | | 3.19 | 3.13 | 2.74 | 0.10 | 3.51 | 3.27 | 3.35 | 0.17 |
| | mean | | | 1.4 | | | | 3.0 | | | | 3.4 | |
| 120 | average | | | 0.1 | | | | 0.2 | | | | 0.1 | |
| | mean colonies | 32.67 | 50.67 | 29.00 | 31.00 | 39.00 | 56.33 | 94.33 | 133.00 | 14.00 | 31.00 | 18.67 | 159.33 |
| | stdev | 2.52 | 6.11 | 6.08 | 11.14 | 4.36 | 11.37 | 4.51 | 1.00 | 1.00 | 10.15 | 2.52 | 67.66 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-6 | 10^-3 | 10^-3 | 10^-3 | 10^-6 | 10^-3 | 10^-3 | 10^-3 | 10^-6 |
| | CFU | 32666.67 | 50666.67 | 29000.00 | 3100000.00 | 3900.00 | 5633.33 | 9433.33 | 13300000.00 | 1400.00 | 3100.00 | 1866.67 | 15933333.33 |
| | $Log_{10}(N_o/N)$ | 1.87 | 1.67 | 1.93 | | 3.74 | 3.55 | 3.33 | 0.19 | 4.15 | 3.81 | 4.02 | 0.12 |
| | mean | | | 1.8 | | | | 3.5 | | | | 4.0 | |
| 240 | average | | | 0.1 | | | | 0.2 | | | | 0.2 | |
| | mean colonies | 165.33 | 216.00 | 136.00 | 31.67 | 18.00 | 33.67 | 61.00 | 132.67 | 63.00 | 124.33 | 97.00 | 168.00 |
| | stdev | 16.74 | 21.66 | 9.64 | 15.50 | 1.73 | 8.08 | 4.36 | 17.62 | 7.94 | 13.01 | 24.43 | 44.84 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-6 | 10^-3 | 10^-3 | 10^-3 | 10^-6 | 10^-2 | 10^-2 | 10^-2 | 10^-6 |
| | CFU | 16533.33 | 21600.00 | 13600.00 | 3166666.67 | 1800.00 | 3366.67 | 6100.00 | 13266666.67 | 630.00 | 1243.33 | 970.00 | 16800000.00 |
| | $Log_{10}(N_o/N)$ | 2.17 | 2.04 | 2.26 | | 4.08 | 3.78 | 3.52 | 0.19 | 4.50 | 4.20 | 4.31 | 0.09 |
| | mean | | | 2.2 | | | | 3.8 | | | | 4.3 | |
| 360 | average | | | 0.1 | | | | 0.3 | | | | 0.2 | |

Table C 5: Effect of Gallic acid on *S. aureus* growth

| contact time (min) | samples | gallic acid 1MIC | | | | gallic acid 2MIC | | | | gallic acid 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | mean colonies | 156.00 | 144.33 | 153.33 | 156.00 | 159.67 | 158.00 | 158.33 | 163.00 | 136.33 | 145.67 | 150.00 | 147.67 |
| | stdev | 14.73 | 7.57 | 0.58 | 2.65 | 10.50 | 7.21 | 2.08 | 6.08 | 13.32 | 9.45 | 18.52 | 24.54 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1560000.00 | 1443333.33 | 1533333.33 | 1560000.00 | 1596666.67 | 1580000.00 | 1583333.33 | 1630000.00 | 1363333.33 | 1456666.67 | 1500000.00 | 1476666.67 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | |
| | mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 0 | STDEV | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | mean colonies | 147.00 | 152.67 | 146.67 | 150.67 | 145.00 | 133.00 | 128.33 | 149.00 | 103.33 | 85.67 | 87.00 | 132.33 |
| | stdev | 12.53 | 34.02 | 8.02 | 18.50 | 18.03 | 16.52 | 18.72 | 4.36 | 5.13 | 13.65 | 8.89 | 10.79 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1470000.00 | 1526666.67 | 1466666.67 | 1506666.67 | 1450000.00 | 1330000.00 | 1283333.33 | 1490000.00 | 1033333.33 | 856666.67 | 870000.00 | 1323333.33 |
| | $Log_{10}(N_o/N)$ | 0.03 | -0.02 | 0.02 | | 0.04 | 0.07 | 0.09 | | 0.12 | 0.23 | 0.24 | |
| | mean | 0.0 | | | | 0.1 | | | | 0.2 | | | |
| 15 | STDEV | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| | mean colonies | 144.50 | 131.00 | 129.67 | 150.33 | 120.00 | 103.67 | 116.67 | 158.00 | 98.00 | 76.67 | 80.00 | 151.33 |
| | stdev | 9.29 | 22.61 | 17.21 | 1.53 | 1.73 | 12.90 | 4.16 | 10.15 | 11.59 | 4.73 | 4.58 | 2.08 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1445000.00 | 1310000.00 | 1296666.67 | 1503333.33 | 1200000.00 | 1036666.67 | 1166666.67 | 1580000.00 | 980000.00 | 766666.67 | 800000.00 | 1513333.33 |
| | $Log_{10}(N_o/N)$ | 0.03 | 0.04 | 0.07 | | 0.12 | 0.18 | 0.13 | | 0.14 | 0.28 | 0.27 | |
| | mean | 0.0 | | | | 0.1 | | | | 0.2 | | | |
| 30 | STDEV | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| | mean colonies | 147.00 | 152.67 | 146.67 | 150.67 | 98.00 | 101.33 | 101.67 | 131.50 | 94.67 | 75.33 | 81.67 | 145.67 |
| | stdev | 12.53 | 34.02 | 8.02 | 18.50 | 27.30 | 9.07 | 0.58 | 7.64 | 35.84 | 13.80 | 5.69 | 21.13 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1470000.00 | 1526666.67 | 1466666.67 | 1506666.67 | 980000.00 | 1013333.33 | 1016666.67 | 1315000.00 | 946666.67 | 753333.33 | 816666.67 | 1456666.67 |
| | $Log_{10}(N_o/N)$ | 0.03 | -0.02 | 0.02 | | 0.21 | 0.19 | 0.19 | | 0.16 | 0.29 | 0.26 | |
| | mean | 0.0 | | | | 0.2 | | | | 0.2 | | | |
| 60 | STDEV | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| | mean colonies | 119.67 | 108.00 | 117.67 | 147.00 | 107.50 | 111.33 | 117.50 | 137.50 | 97.00 | 81.67 | 92.33 | 138.00 |
| | stdev | 4.73 | 2.00 | 1.53 | 12.53 | 22.07 | 16.01 | 7.94 | 5.00 | 4.36 | 11.93 | 8.39 | 8.50 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1196666.67 | 1080000.00 | 1176666.67 | 1470000.00 | 1075000.00 | 1113333.33 | 1175000.00 | 1375000.00 | 970000.00 | 816666.67 | 923333.33 | 1380000.00 |
| | $Log_{10}(N_o/N)$ | 0.12 | 0.13 | 0.11 | | 0.17 | 0.15 | 0.13 | | 0.15 | 0.25 | 0.21 | |
| | mean | 0.1 | | | | 0.2 | | | | 0.2 | | | |
| 120 | STDEV | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| | mean colonies | 118.00 | 112.33 | 128.67 | 159.50 | 87.67 | 105.67 | 96.67 | 134.00 | 60.33 | 80.00 | 69.00 | 138.50 |
| | stdev | 33.65 | 15.04 | 21.55 | 6.08 | 8.33 | 5.03 | 9.02 | 2.31 | 8.08 | 10.82 | 12.77 | 28.92 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1180000.00 | 1123333.33 | 1286666.67 | 1595000.00 | 876666.67 | 1056666.67 | 966666.67 | 1340000.00 | 603333.33 | 800000.00 | 690000.00 | 1385000.00 |
| | $Log_{10}(N_o/N)$ | 0.12 | 0.11 | 0.08 | | 0.26 | 0.17 | 0.21 | | 0.35 | 0.26 | 0.34 | |
| | mean | 0.1 | | | | 0.2 | | | | 0.3 | | | |
| 240 | STDEV | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| | mean colonies | 118.67 | 110.33 | 118.67 | 167.00 | 96.00 | 87.00 | 111.00 | 127.67 | 74.33 | 74.67 | 68.67 | 122.67 |
| | stdev | 19.86 | 3.21 | 2.31 | 3.61 | 14.93 | 16.46 | 16.52 | 3.21 | 14.84 | 10.12 | 1.53 | 14.19 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1186666.67 | 1103333.33 | 1186666.67 | 1670000.00 | 960000.00 | 870000.00 | 1110000.00 | 1276666.67 | 743333.33 | 746666.67 | 686666.67 | 1226666.67 |
| | $Log_{10}(N_o/N)$ | 0.12 | 0.12 | 0.11 | | 0.22 | 0.26 | 0.15 | | 0.26 | 0.29 | 0.34 | |
| | mean | 0.1 | | | | 0.2 | | | | 0.3 | | | |
| 360 | STDEV | 0.0 | | | | 0.1 | | | | 0.0 | | | |

Table C 6: Effect of catechin on *S. aureus* growth

| contact time (min) | samples | catechin 1MIC | | | | catechin 2MIC | | | | catechin 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | mean colonies | 270.67 | 278.67 | 272.67 | 273.00 | 196.33 | 207.67 | 205.67 | 204.33 | 207.67 | 250.33 | 231.67 | 227.00 |
| | stdev | 4.04 | 13.01 | 17.30 | 34.04 | 21.83 | 34.20 | 17.21 | 5.13 | 10.36 | 9.50 | 8.08 | 36.29 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 2706666.67 | 2786666.67 | 2726666.67 | 2730000.00 | 1963333.33 | 2076666.67 | 2056666.67 | 2043333.33 | 2076666.67 | 2503333.33 | 2316666.67 | 2270000.00 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 259.50 | 272.67 | 242.67 | 270.67 | 157.67 | 189.67 | 174.67 | 223.33 | 143.33 | 143.00 | 92.67 | 179.33 |
| | stdev | 13.03 | 14.78 | 27.68 | 66.79 | 11.59 | 20.50 | 11.37 | 92.18 | 2.89 | 32.14 | 10.02 | 61.08 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 2595000.00 | 2726666.67 | 2426666.67 | 2706666.67 | 1576666.67 | 1896666.67 | 1746666.67 | 2233333.33 | 1433333.33 | 1430000.00 | 926666.67 | 1793333.33 |
| | $Log_{10}(N_o/N)$ | 0.02 | 0.01 | 0.05 | | 0.10 | 0.04 | 0.07 | | 0.16 | 0.24 | 0.40 | |
| | mean | | | 0.0 | | | | 0.1 | | | | 0.3 | |
| 15 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| | mean colonies | 245.50 | 249.00 | 257.67 | 283.33 | 167.00 | 193.33 | 182.00 | 222.00 | 107.00 | 146.67 | 155.33 | 185.67 |
| | stdev | 9.50 | 6.80 | 20.21 | 58.23 | 53.67 | 18.88 | 36.01 | 10.44 | 37.54 | 30.14 | 14.57 | 35.00 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 2455000.00 | 2490000.00 | 2576666.67 | 2833333.33 | 1670000.00 | 1933333.33 | 1820000.00 | 2220000.00 | 1070000.00 | 1466666.67 | 1553333.33 | 1856666.67 |
| | $Log_{10}(N_o/N)$ | 0.04 | 0.05 | 0.02 | | 0.07 | 0.03 | 0.05 | | 0.29 | 0.23 | 0.17 | |
| | mean | | | 0.0 | | | | 0.1 | | | | 0.2 | |
| 30 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| | mean colonies | 216.33 | 221.67 | 234.33 | 283.33 | 176.00 | 205.67 | 182.00 | 222.00 | 154.00 | 204.00 | 114.33 | 196.00 |
| | stdev | 25.03 | 4.73 | 2.89 | 58.23 | 53.67 | 18.88 | 36.01 | 10.44 | 86.49 | 33.17 | 49.57 | 35.59 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 2163333.33 | 2216666.67 | 2343333.33 | 2833333.33 | 1760000.00 | 2056666.67 | 1820000.00 | 2220000.00 | 1540000.00 | 2040000.00 | 1143333.33 | 1960000.00 |
| | $Log_{10}(N_o/N)$ | 0.10 | 0.10 | 0.07 | | 0.05 | 0.00 | 0.05 | | 0.13 | 0.09 | 0.31 | |
| | mean | | | 0.1 | | | | 0.0 | | | | 0.2 | |
| 60 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| | mean colonies | 282.00 | 249.33 | 263.67 | 304.67 | 167.67 | 198.00 | 185.00 | 211.33 | 186.67 | 193.33 | 145.33 | 196.00 |
| | stdev | 51.97 | 34.12 | 29.70 | 13.80 | 34.82 | 16.00 | 3.00 | 25.70 | 86.49 | 33.17 | 49.57 | 35.59 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 2820000.00 | 2493333.33 | 2636666.67 | 3046666.67 | 1676666.67 | 1980000.00 | 1850000.00 | 2113333.33 | 1866666.67 | 1933333.33 | 1453333.33 | 1960000.00 |
| | $Log_{10}(N_o/N)$ | -0.02 | 0.05 | 0.01 | | 0.07 | 0.02 | 0.05 | | 0.05 | 0.11 | 0.20 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| 120 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| | mean colonies | 272.00 | 254.33 | 266.00 | 298.00 | 184.33 | 209.33 | 211.00 | 217.50 | 212.67 | 146.33 | 179.67 | 180.50 |
| | stdev | 12.12 | 12.50 | 5.57 | 10.39 | 8.08 | 8.33 | 10.54 | 13.80 | 31.53 | 50.93 | 0.58 | 9.81 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 2720000.00 | 2543333.33 | 2660000.00 | 2980000.00 | 1843333.33 | 2093333.33 | 2110000.00 | 2175000.00 | 2126666.67 | 1463333.33 | 1796666.67 | 1805000.00 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.04 | 0.01 | | 0.03 | 0.00 | -0.01 | | -0.01 | 0.23 | 0.11 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| 240 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| | mean colonies | 246.33 | 245.33 | 223.50 | 282.00 | 237.67 | 272.67 | 269.33 | 216.67 | 157.67 | 130.00 | 130.00 | 176.33 |
| | stdev | 14.57 | 5.69 | 31.82 | 13.00 | 10.69 | 44.09 | 1.15 | 50.95 | 28.57 | 27.07 | 19.52 | 5.13 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 2463333.33 | 2453333.33 | 2235000.00 | 2820000.00 | 2376666.67 | 2726666.67 | 2693333.33 | 2166666.67 | 1576666.67 | 1300000.00 | 1300000.00 | 1763333.33 |
| | $Log_{10}(N_o/N)$ | 0.04 | 0.06 | 0.09 | | -0.08 | -0.12 | -0.12 | | 0.12 | 0.28 | 0.25 | |
| | mean | | | 0.1 | | | | -0.1 | | | | 0.2 | |
| 360 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |

Table C 7: Effect of Epicatechin on *S. aureus* growth

| contact time (min) | sample | Epicatechin 1xMIC | | | | Epicatechin 2xMIC | | | | Epicatechin 4xMIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | mean colonies | 189.67 | 176.00 | 186.67 | 185.33 | 204.33 | 212.33 | 211.67 | 207.67 | 184.33 | 184.33 | 201.33 | 185.33 |
| | stdev | 16.26 | 10.82 | 5.69 | 9.29 | 4.51 | 19.66 | 15.14 | 19.40 | 5.69 | 5.51 | 10.97 | 13.80 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1896666.67 | 1760000.00 | 1866666.67 | 1853333.33 | 2043333.33 | 2123333.33 | 2116666.67 | 2076666.67 | 1843333.33 | 1843333.33 | 2013333.33 | 1853333.33 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | |
| | Mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 182.67 | 120.67 | 167.67 | 166.67 | 202.00 | 197.67 | 202.67 | 223.00 | 170.00 | 187.00 | 178.00 | 185.33 |
| | stdev | 24.54 | 28.88 | 23.86 | 29.69 | 21.07 | 14.64 | 3.79 | 49.24 | 56.31 | 14.73 | 10.15 | 13.80 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1826666.67 | 1206666.67 | 1676666.67 | 1666666.67 | 2020000.00 | 1976666.67 | 2026666.67 | 2230000.00 | 1700000.00 | 1870000.00 | 1780000.00 | 1853333.33 |
| | $Log_{10}(N_o/N)$ | 0.02 | 0.16 | 0.05 | | 0.00 | 0.03 | 0.02 | | 0.04 | -0.01 | 0.05 | |
| | Mean | | | 0.1 | | | | 0.0 | | | | 0.0 | |
| 15 | STDEV | | | 0.1 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 176.00 | 105.00 | 174.00 | 177.67 | 189.50 | 200.00 | 208.33 | 244.00 | 228.00 | 247.67 | 226.33 | 190.33 |
| | stdev | 13.20 | 19.97 | 6.00 | 17.93 | 7.23 | 1.73 | 1.53 | 22.72 | 18.00 | 15.18 | 5.51 | 7.51 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1760000.00 | 1050000.00 | 1740000.00 | 1776666.67 | 1895000.00 | 2000000.00 | 2083333.33 | 2440000.00 | 2280000.00 | 2476666.67 | 2263333.33 | 1903333.33 |
| | $Log_{10}(N_o/N)$ | 0.03 | 0.22 | 0.03 | | 0.03 | 0.03 | 0.01 | | -0.09 | -0.13 | -0.05 | |
| | Mean | | | 0.1 | | | | 0.0 | | | | -0.1 | |
| 30 | STDEV | | | 0.1 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 132.67 | 143.67 | 151.00 | 180.33 | 220.00 | 221.67 | 206.33 | 238.67 | 163.00 | 242.00 | 231.00 | 208.33 |
| | stdev | 23.12 | 6.43 | 11.53 | 5.13 | 13.01 | 5.69 | 5.51 | 26.73 | 32.23 | 6.93 | 4.58 | 4.51 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1326666.67 | 1436666.67 | 1510000.00 | 1803333.33 | 2200000.00 | 2216666.67 | 2063333.33 | 2386666.67 | 1630000.00 | 2420000.00 | 2310000.00 | 2083333.33 |
| | $Log_{10}(N_o/N)$ | 0.16 | 0.09 | 0.09 | | -0.03 | -0.02 | 0.01 | | 0.05 | -0.12 | -0.06 | |
| | Mean | | | 0.1 | | | | 0.0 | | | | 0.0 | |
| 60 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| | mean colonies | 78.00 | 101.33 | 114.33 | 174.33 | 116.00 | 149.33 | 144.67 | 163.67 | 230.67 | 230.67 | 229.67 | 218.33 |
| | stdev | 19.05 | 31.90 | 42.78 | 17.79 | 30.81 | 7.23 | 14.05 | 24.01 | 4.04 | 4.04 | 5.51 | 17.24 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 780000.00 | 1013333.33 | 1143333.33 | 1743333.33 | 1160000.00 | 1493333.33 | 1446666.67 | 1636666.67 | 2306666.67 | 2306666.67 | 2296666.67 | 2183333.33 |
| | $Log_{10}(N_o/N)$ | 0.39 | 0.24 | 0.21 | | 0.25 | 0.15 | 0.17 | | -0.10 | -0.10 | -0.06 | |
| | Mean | | | 0.3 | | | | 0.2 | | | | -0.1 | |
| 120 | STDEV | | | 0.1 | | | | 0.1 | | | | 0.0 | |
| | mean colonies | 87.67 | 134.33 | 137.33 | 174.50 | 174.33 | 157.00 | 166.67 | 188.50 | 191.00 | 198.33 | 206.00 | 225.50 |
| | stdev | 6.11 | 17.79 | 9.24 | 20.55 | 1.15 | 2.65 | 28.38 | 41.79 | 47.70 | 67.02 | 21.17 | 5.69 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 876666.67 | 1343333.33 | 1373333.33 | 1745000.00 | 1743333.33 | 1570000.00 | 1666666.67 | 1885000.00 | 1910000.00 | 1983333.33 | 2060000.00 | 2255000.00 |
| | $Log_{10}(N_o/N)$ | 0.34 | 0.12 | 0.13 | | 0.07 | 0.13 | 0.10 | | -0.02 | -0.03 | -0.01 | |
| | Mean | | | 0.2 | | | | 0.1 | | | | 0.0 | |
| 240 | STDEV | | | 0.1 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 129.00 | 131.00 | 118.33 | 184.00 | 176.00 | 180.00 | 186.67 | 167.67 | 176.67 | 166.00 | 184.67 | 202.67 |
| | stdev | 35.59 | 36.59 | 10.26 | 14.73 | 5.29 | 18.03 | 11.68 | 10.02 | 11.59 | 32.36 | 52.94 | 38.68 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1290000.00 | 1310000.00 | 1183333.33 | 1840000.00 | 1760000.00 | 1800000.00 | 1866666.67 | 1676666.67 | 1766666.67 | 1660000.00 | 1846666.67 | 2026666.67 |
| | $Log_{10}(N_o/N)$ | 0.17 | 0.13 | 0.20 | | 0.06 | 0.07 | 0.05 | | 0.02 | 0.05 | 0.04 | |
| | Mean | | | 0.2 | | | | 0.1 | | | | 0.0 | |
| 360 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |

**Appendix C3: Raw data for *E. coli* disinfection**

Table C 8: Effect of banana extracts on *E. coli* growth

| | | FD3hr 1MIC | | | | FD3hr 2MIC | | | | FD3hr 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| contact time (min | sample | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | mean colonies | 76.33 | 79.33 | 76.67 | 84.33 | 99.33 | 80.00 | 96.33 | 87.33 | 73.00 | 71.67 | 81.00 | 78.67 |
| | stdev | 28.54 | 3.79 | 14.47 | 13.58 | 17.90 | 16.46 | 4.62 | 17.93 | 4.36 | 3.51 | 9.85 | 5.51 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 763333.33 | 793333.33 | 766666.67 | 843333.33 | 993333.33 | 800000.00 | 963333.33 | 873333.33 | 730000.00 | 716666.67 | 810000.00 | 786666.67 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 63.33 | 86.67 | 75.33 | 84.33 | 77.33 | 78.67 | 87.33 | 97.67 | 48.33 | 51.67 | 53.67 | 80.33 |
| | stdev | 42.36 | 10.69 | 8.39 | 13.58 | 9.07 | 9.45 | 22.90 | 24.99 | 7.77 | 6.43 | 3.21 | 5.13 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 633333.33 | 866666.67 | 753333.33 | 843333.33 | 773333.33 | 786666.67 | 873333.33 | 976666.67 | 483333.33 | 516666.67 | 536666.67 | 803333.33 |
| | $Log_{10}(N_o/N)$ | 0.08 | -0.04 | 0.01 | | 0.11 | 0.01 | 0.04 | | 0.18 | 0.14 | 0.18 | |
| | mean | | | 0.0 | | | | 0.1 | | | | 0.2 | |
| 30 | STDEV | | | 0.1 | | | | 0.1 | | | | 0.0 | |
| | mean colonies | 67.00 | 58.67 | 51.67 | 84.00 | 69.33 | 71.33 | 74.33 | 81.67 | 59.33 | 49.00 | 52.67 | 73.67 |
| | stdev | 4.36 | 14.74 | 9.02 | 23.81 | 7.77 | 8.08 | 1.15 | 15.95 | 13.58 | 9.54 | 2.08 | 2.52 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 670000.00 | 586666.67 | 516666.67 | 840000.00 | 693333.33 | 713333.33 | 743333.33 | 816666.67 | 593333.33 | 490000.00 | 526666.67 | 736666.67 |
| | $Log_{10}(N_o/N)$ | 0.06 | 0.13 | 0.17 | | 0.16 | 0.05 | 0.11 | | 0.09 | 0.17 | 0.19 | |
| | mean | | | 0.1 | | | | 0.1 | | | | 0.1 | |
| 60 | STDEV | | | 0.1 | | | | 0.1 | | | | 0.1 | |
| | mean colonies | 59.33 | 71.00 | 71.33 | 80.00 | 78.00 | 67.33 | 82.33 | 94.00 | 59.00 | 50.33 | 62.33 | 107.00 |
| | stdev | 19.04 | 7.94 | 7.57 | 26.23 | 23.43 | 5.86 | 3.21 | 12.53 | 9.85 | 4.93 | 10.21 | 17.78 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 593333.33 | 710000.00 | 713333.33 | 800000.00 | 780000.00 | 673333.33 | 823333.33 | 940000.00 | 590000.00 | 503333.33 | 623333.33 | 1070000.00 |
| | $Log_{10}(N_o/N)$ | 0.11 | 0.05 | 0.03 | | 0.11 | 0.07 | 0.07 | | 0.09 | 0.15 | 0.11 | |
| | mean | | | 0.1 | | | | 0.1 | | | | 0.1 | |
| 120 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 99.00 | 84.67 | 91.33 | 77.67 | 65.00 | 61.00 | 72.67 | 79.33 | 47.33 | 49.67 | 49.33 | 79.00 |
| | stdev | 20.66 | 6.81 | 17.56 | 17.21 | 14.73 | 5.20 | 4.93 | 4.93 | 4.73 | 2.08 | 3.06 | 4.00 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 990000.00 | 846666.67 | 913333.33 | 776666.67 | 650000.00 | 610000.00 | 726666.67 | 793333.33 | 473333.33 | 496666.67 | 493333.33 | 790000.00 |
| | $Log_{10}(N_o/N)$ | -0.11 | -0.03 | -0.08 | | 0.18 | 0.12 | 0.12 | | 0.19 | 0.16 | 0.22 | |
| | mean | | | -0.1 | | | | 0.1 | | | | 0.2 | |
| 240 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 90.33 | 82.33 | 84.67 | 64.67 | 46.67 | 45.33 | 52.00 | 64.67 | 40.67 | 40.33 | 39.33 | 73.00 |
| | stdev | 12.90 | 6.43 | 8.39 | 11.50 | 4.73 | 10.02 | 11.53 | 11.24 | 4.51 | 6.81 | 8.08 | 6.08 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 903333.33 | 823333.33 | 846666.67 | 646666.67 | 466666.67 | 453333.33 | 520000.00 | 646666.67 | 406666.67 | 403333.33 | 393333.33 | 730000.00 |
| | $Log_{10}(N_o/N)$ | -0.07 | -0.02 | -0.04 | | 0.33 | 0.25 | 0.27 | | 0.25 | 0.25 | 0.31 | |
| | mean | | | 0.0 | | | | 0.3 | | | | 0.3 | |
| 360 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |

Table C 9: Effect of Epigallocatechin gallate on *E. coli* growth

| | | EGCg 0.5 MIC | | | | EGCg 1MIC | | | | EGCg 2MIC | | | | EGCg 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Contact time (min) | samples | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | mean colonies | 101.33 | 88.00 | 90.00 | 89.33 | 67.00 | 69.00 | 67.00 | 67.00 | 102.00 | 103.33 | 103.00 | 108.67 | 101.33 | 116.33 | 110.67 | 113.33 |
| | stdev | 3.06 | 5.00 | 7.94 | 6.51 | 16.20 | 15.31 | 7.21 | 2.08 | 15.39 | 22.37 | 11.27 | 23.80 | 3.51 | 5.03 | 9.29 | 6.51 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-6 | 10^-6 | 10^-6 | 10^-6 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1013333.33 | 880000.00 | 900000.00 | 893333.33 | 670000.00 | 690000.00 | 670000.00 | 670000.00 | 1020000.00 | 1033333.33 | 1030000.00 | 1086666.67 | 1013333.33 | 1163333.33 | 1106666.67 | 1133333.33 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 94.00 | 93.00 | 85.67 | 101.67 | 63.67 | 68.33 | 62.67 | 67.00 | 97.67 | 69.00 | 71.33 | 110.67 | 84.33 | 103.33 | 73.00 | 114.33 |
| | stdev | 8.89 | 8.72 | 9.50 | 5.51 | 3.51 | 19.55 | 7.02 | 4.36 | 28.75 | 4.00 | 6.43 | 23.86 | 29.40 | 36.83 | 14.18 | 28.75 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 940000.00 | 930000.00 | 856666.67 | 1016666.67 | 636666.67 | 683333.33 | 626666.67 | 670000.00 | 976666.67 | 690000.00 | 713333.33 | 1106666.67 | 843333.33 | 1033333.33 | 730000.00 | 1143333.33 |
| | $Log_{10}(N_o/N)$ | 0.03 | -0.02 | 0.02 | | 0.02 | 0.00 | 0.03 | | 0.02 | 0.18 | 0.16 | | 0.08 | 0.05 | 0.18 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.1 | | | | 0.1 | |
| 30 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | | | | 0.1 | |
| | mean colonies | 100.00 | 91.00 | 103.67 | 104.33 | 55.67 | 76.33 | 68.33 | 81.67 | 75.00 | 89.00 | 95.00 | 113.00 | 100.00 | 117.33 | 95.33 | 100.33 |
| | stdev | 13.00 | 9.00 | 14.29 | 19.86 | 3.51 | 7.02 | 8.08 | 14.22 | 11.14 | 15.72 | 13.53 | 16.87 | 23.07 | 31.34 | 8.08 | 10.50 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1000000.00 | 910000.00 | 1036666.67 | 1043333.33 | 556666.67 | 763333.33 | 683333.33 | 816666.67 | 750000.00 | 890000.00 | 950000.00 | 1130000.00 | 1000000.00 | 1173333.33 | 953333.33 | 1003333.33 |
| | $Log_{10}(N_o/N)$ | 0.01 | -0.01 | -0.06 | | 0.08 | -0.04 | -0.01 | | 0.13 | 0.06 | 0.04 | | 0.01 | 0.00 | 0.06 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.1 | | | | 0.0 | |
| 60 | STDEV | | | 0.0 | | | | 0.1 | | | | 0.1 | | | | 0.0 | |
| | mean colonies | 93.67 | 95.33 | 72.67 | 104.67 | 56.67 | 70.67 | 79.33 | 76.33 | 112.00 | 98.67 | 78.67 | 110.67 | 103.00 | 82.67 | 106.33 | 104.33 |
| | stdev | 22.55 | 6.03 | 9.29 | 14.84 | 8.33 | 3.21 | 8.14 | 4.73 | 16.64 | 3.79 | 15.50 | 8.14 | 28.79 | 21.39 | 51.39 | 9.29 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 936666.67 | 953333.33 | 726666.67 | 1046666.67 | 566666.67 | 706666.67 | 793333.33 | 763333.33 | 1120000.00 | 986666.67 | 786666.67 | 1106666.67 | 1030000.00 | 826666.67 | 1063333.33 | 1043333.33 |
| | $Log_{10}(N_o/N)$ | 0.03 | -0.03 | 0.09 | | 0.07 | -0.01 | -0.07 | | -0.04 | 0.02 | 0.12 | | -0.01 | 0.15 | 0.02 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| 120 | STDEV | | | 0.1 | | | | 0.1 | | | | 0.1 | | | | 0.1 | |
| | mean colonies | 86.00 | 65.00 | 77.33 | 100.00 | 39.33 | 41.67 | 50.00 | 61.00 | 79.33 | 60.33 | 62.33 | 106.00 | 82.00 | 60.67 | 52.67 | 107.67 |
| | stdev | 2.00 | 10.44 | 12.10 | 3.00 | 1.15 | 10.02 | 7.00 | 8.19 | 31.56 | 5.13 | 1.53 | 17.09 | 24.27 | 3.21 | 10.26 | 18.15 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 860000.00 | 650000.00 | 773333.33 | 1000000.00 | 393333.33 | 416666.67 | 500000.00 | 610000.00 | 793333.33 | 603333.33 | 623333.33 | 1060000.00 | 820000.00 | 606666.67 | 526666.67 | 1076666.67 |
| | $Log_{10}(N_o/N)$ | 0.07 | 0.13 | 0.07 | | 0.23 | 0.22 | 0.13 | | 0.11 | 0.23 | 0.22 | | 0.09 | 0.28 | 0.32 | |
| | mean | | | 0.1 | | | | 0.2 | | | | 0.2 | | | | 0.2 | |
| 240 | STDEV | | | 0.1 | | | | 0.1 | | | | 0.1 | | | | 0.1 | |
| | mean colonies | 58.33 | 60.00 | 62.00 | 89.67 | 71.00 | 77.00 | 72.67 | 63.67 | 150.67 | 120.67 | 125.67 | 119.33 | 42.00 | 65.33 | 49.33 | 102.67 |
| | stdev | 5.69 | 3.00 | 5.29 | 9.71 | 1.00 | 1.73 | 6.81 | 29.01 | 9.81 | 14.36 | 20.31 | 11.02 | 15.39 | 12.86 | 8.96 | 12.90 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-4 | 10^-4 | 10^-4 | 10^-5 | 10^-4 | 10^-4 | 10^-4 | 10^-5 |
| | cfu | 583333.33 | 600000.00 | 620000.00 | 896666.67 | 71000.00 | 77000.00 | 72666.67 | 636666.67 | 150666.67 | 120666.67 | 125666.67 | 1193333.33 | 42000.00 | 65333.33 | 49333.33 | 1026666.67 |
| | $Log_{10}(N_o/N)$ | 0.24 | 0.17 | 0.16 | | 0.97 | 0.95 | 0.96 | | 0.83 | 0.93 | 0.91 | | 1.38 | 1.25 | 1.35 | |
| | mean | | | 0.2 | | | | 0.9 | | | | 0.9 | | | | 1.3 | |
| 360 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | | | | 0.1 | |

Table C 10: Effect of gallic acid on *E. coli* growth

| | | Gallic acid 1MIC | | | | Gallic acid 2MIC | | | | Gallic acid 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| contact time(min) | sample | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | mean colonies | 134.67 | 115.33 | 153.00 | 130.00 | 105.33 | 100.00 | 95.67 | 99.33 | 96.33 | 89.00 | 101.67 | 100.00 |
| | stdev | 7.02 | 13.01 | 18.68 | 8.54 | 3.51 | 10.82 | 24.58 | 17.62 | 26.03 | 12.00 | 1.53 | 15.72 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1346666.67 | 1153333.33 | 1530000.00 | 1300000.00 | 1053333.33 | 1000000.00 | 956666.67 | 993333.33 | 963333.33 | 890000.00 | 1016666.67 | 1000000.00 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 119.67 | 131.67 | 117.00 | 125.67 | 98.67 | 99.33 | 116.33 | 101.33 | 79.33 | 99.33 | 103.00 | 106.00 |
| | stdev | 17.79 | 9.29 | 29.51 | 10.02 | 11.68 | 3.06 | 6.11 | 8.62 | 6.66 | 14.01 | 6.08 | 23.90 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1196666.67 | 1316666.67 | 1170000.00 | 1256666.67 | 986666.67 | 993333.33 | 1163333.33 | 1013333.33 | 793333.33 | 993333.33 | 1030000.00 | 1060000.00 |
| | $Log_{10}(N_o/N)$ | 0.05 | -0.06 | 0.12 | | 0.03 | 0.00 | -0.08 | | 0.08 | -0.05 | -0.01 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 15 | STDEV | | | 0.1 | | | | 0.1 | | | | 0.1 | |
| | mean colonies | 119.00 | 117.33 | 112.67 | 122.00 | 96.00 | 106.00 | 104.33 | 106.67 | 114.33 | 98.00 | 86.67 | 115.00 |
| | stdev | 12.29 | 13.20 | 5.03 | 7.81 | 16.00 | 11.00 | 22.01 | 17.62 | 2.08 | 4.58 | 22.59 | 27.22 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1190000.00 | 1173333.33 | 1126666.67 | 1220000.00 | 960000.00 | 1060000.00 | 1043333.33 | 1066666.67 | 1143333.33 | 980000.00 | 866666.67 | 1150000.00 |
| | $Log_{10}(N_o/N)$ | 0.05 | -0.01 | 0.13 | | 0.04 | -0.03 | -0.04 | | -0.07 | -0.04 | 0.07 | |
| | mean | | | 0.1 | | | | 0.0 | | | | 0.0 | |
| 30 | STDEV | | | 0.1 | | | | 0.0 | | | | 0.1 | |
| | mean colonies | 109.00 | 119.00 | 107.67 | 117.00 | 124.67 | 125.00 | 129.33 | 136.33 | 96.00 | 84.00 | 83.00 | 91.33 |
| | stdev | 3.00 | 13.45 | 4.16 | 1.73 | 18.15 | 33.15 | 13.32 | 26.01 | 13.00 | 22.61 | 14.00 | 10.69 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1090000.00 | 1190000.00 | 1076666.67 | 1170000.00 | 1246666.67 | 1250000.00 | 1293333.33 | 1363333.33 | 960000.00 | 840000.00 | 830000.00 | 913333.33 |
| | $Log_{10}(N_o/N)$ | 0.05 | 0.03 | 0.12 | | -0.07 | -0.10 | -0.13 | | 0.00 | 0.03 | 0.09 | |
| | mean | | | 0.1 | | | | -0.1 | | | | 0.0 | |
| 60 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean colonies | 114.67 | 103.00 | 109.00 | 120.00 | 93.00 | 92.67 | 113.67 | 101.67 | 105.33 | 100.67 | 98.00 | 102.67 |
| | stdev | 6.03 | 5.20 | 5.20 | 7.00 | 10.39 | 10.69 | 24.54 | 6.81 | 19.40 | 10.69 | 18.25 | 23.25 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1146666.67 | 1030000.00 | 1090000.00 | 1200000.00 | 930000.00 | 926666.67 | 1136666.67 | 1016666.67 | 1053333.33 | 1006666.67 | 980000.00 | 1026666.67 |
| | $Log_{10}(N_o/N)$ | 0.07 | 0.05 | 0.15 | | 0.05 | 0.03 | -0.07 | | -0.04 | -0.05 | 0.02 | |
| | mean | | | 0.1 | | | | 0.0 | | | | 0.0 | |
| 120 | STDEV | | | 0.1 | | | | 0.1 | | | | 0.0 | |
| | mean colonies | 108.67 | 112.00 | 110.67 | 112.67 | 94.67 | 118.00 | 130.33 | 138.33 | 81.33 | 80.00 | 74.00 | 94.67 |
| | stdev | 10.02 | 16.52 | 7.37 | 9.29 | 7.37 | 21.79 | 10.69 | 21.22 | 8.62 | 6.56 | 6.24 | 10.69 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1086666.67 | 1120000.00 | 1106666.67 | 1126666.67 | 946666.67 | 1180000.00 | 1303333.33 | 1383333.33 | 813333.33 | 800000.00 | 740000.00 | 946666.67 |
| | $Log_{10}(N_o/N)$ | 0.09 | 0.01 | 0.14 | | 0.05 | -0.07 | -0.13 | | 0.07 | 0.05 | 0.14 | |
| | mean | | | 0.1 | | | | -0.1 | | | | 0.1 | |
| 240 | STDEV | | | 0.1 | | | | 0.1 | | | | 0.0 | |
| | mean colonies | 116.00 | 168.67 | 111.00 | 128.00 | 88.33 | 121.67 | 118.33 | 109.00 | 84.00 | 74.67 | 72.33 | 85.00 |
| | stdev | 32.19 | 24.58 | 11.53 | 7.00 | 6.66 | 23.76 | 38.63 | 20.81 | 12.77 | 5.13 | 8.50 | 1.73 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu | 1160000.00 | 1686666.67 | 1110000.00 | 1280000.00 | 883333.33 | 1216666.67 | 1183333.33 | 1090000.00 | 840000.00 | 746666.67 | 723333.33 | 850000.00 |
| | $Log_{10}(N_o/N)$ | 0.06 | -0.17 | 0.14 | | 0.08 | -0.09 | -0.09 | | 0.06 | 0.08 | 0.15 | |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| 360 | STDEV | | | 0.2 | | | | 0.1 | | | | 0.0 | |

**Appendix C4: Raw data for *B. cepacia* disinfection**

Table C 11: Effect of Epigallocatechin gallate on *B. cepacia* growth

| | | EGCg 1MIC | | | | EGCg 2MIC | | | | EGCg 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Replicate 1 | | Replicate 2 | | Replicate 1 | | Replicate 2 | | Replicate 1 | | Replicate 2 | |
| contact time (min) | Sample | sample | Control | sample | Control | sample | Control | sample | Control | sample | Control | sample | Control |
| | Mean colonies | 125.33 | 125.33 | 151.00 | 151.00 | 125.33 | 125.33 | 151.00 | 151.00 | 151.00 | 151.00 | 125.33 | 125.33 |
| | stdev | 5.51 | 5.51 | 6.24 | 6.24 | 5.51 | 5.51 | 6.24 | 6.24 | 6.24 | 6.24 | 5.51 | 5.51 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 125333.33 | 125333.33 | 151000.00 | 151000.00 | 125333.33 | 125333.33 | 151000.00 | 151000.00 | 151000.00 | 151000.00 | 125333.33 | 125333.33 |
| | Log (N0/N) | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 0 | STDEV | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 112.33 | 129.33 | 138.33 | 154.00 | 116.33 | 129.33 | 153.00 | 154.00 | 150.67 | 154.00 | 119.00 | 129.33 |
| | stdev | 2.89 | 2.52 | 9.50 | 2.31 | 6.51 | 2.52 | 10.82 | 2.31 | 12.50 | 2.31 | 4.58 | 2.52 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 112333.33 | 129333.33 | 138333.33 | 154000.00 | 116333.33 | 129333.33 | 153000.00 | 154000.00 | 150666.67 | 154000.00 | 119000.00 | 129333.33 |
| | Log (N0/N) | 0.05 | | 0.04 | | 0.03 | | -0.01 | | 0.00 | | 0.02 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 15 | STDEV | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 123.50 | 128.33 | 143.33 | 162.33 | 120.00 | 128.33 | 145.33 | 162.33 | 158.33 | 162.33 | 111.33 | 128.33 |
| | stdev | 3.54 | 12.42 | 19.86 | 5.69 | 7.81 | 12.42 | 3.21 | 5.69 | 17.21 | 5.69 | 2.08 | 12.42 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 123500.00 | 128333.33 | 143333.33 | 162333.33 | 120000.00 | 128333.33 | 145333.33 | 162333.33 | 158333.33 | 162333.33 | 111333.33 | 128333.33 |
| | Log (N0/N) | 0.01 | | 0.02 | | 0.02 | | 0.02 | | -0.02 | | 0.05 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 30 | STDEV | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| | Mean colonies | 120.00 | 128.33 | 147.00 | 151.67 | 121.67 | 128.33 | 137.33 | 151.67 | 147.33 | 151.67 | 126.33 | 128.33 |
| | stdev | 26.87 | 12.42 | 7.81 | 2.89 | 3.06 | 12.42 | 13.58 | 2.89 | 15.63 | 2.89 | 2.52 | 12.42 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 120000.00 | 128333.33 | 147000.00 | 151666.67 | 121666.67 | 128333.33 | 137333.33 | 151666.67 | 147333.33 | 151666.67 | 126333.33 | 128333.33 |
| | Log (N0/N) | 0.02 | | 0.01 | | 0.01 | | 0.04 | | 0.01 | | 0.00 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 60 | STDEV | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 117.33 | 117.67 | 152.00 | 150.00 | 123.67 | 117.67 | 142.33 | 150.00 | 156.67 | 150.00 | 127.00 | 117.67 |
| | stdev | 9.07 | 5.03 | 4.58 | 1.73 | 5.69 | 5.03 | 23.18 | 1.73 | 14.29 | 1.73 | 7.00 | 5.03 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 117333.33 | 117666.67 | 152000.00 | 150000.00 | 123666.67 | 117666.67 | 142333.33 | 150000.00 | 156666.67 | 150000.00 | 127000.00 | 117666.67 |
| | Log (N0/N) | 0.03 | | 0.00 | | 0.01 | | 0.03 | | -0.02 | | -0.01 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 120 | STDEV | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 110.00 | 125.33 | 154.67 | 147.33 | 123.67 | 125.33 | 152.00 | 147.33 | 152.33 | 147.33 | 128.67 | 125.33 |
| | stdev | 4.36 | 8.39 | 1.15 | 4.04 | 25.15 | 8.39 | 8.72 | 4.04 | 3.21 | 4.04 | 5.51 | 8.39 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 110000.00 | 125333.33 | 154666.67 | 147333.33 | 123666.67 | 125333.33 | 152000.00 | 147333.33 | 152333.33 | 147333.33 | 128666.67 | 125333.33 |
| | Log (N0/N) | 0.06 | | -0.01 | | 0.01 | | 0.00 | | 0.00 | | -0.01 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 240 | STDEV | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 117.67 | 128.00 | 142.00 | 162.33 | 131.00 | 128.00 | 134.67 | 162.33 | 136.67 | 162.33 | 119.33 | 128.00 |
| | stdev | 7.57 | 9.17 | 10.39 | 3.79 | 2.65 | 9.17 | 2.31 | 3.79 | 12.66 | 3.79 | 1.53 | 9.17 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 117666.67 | 128000.00 | 142000.00 | 162333.33 | 131000.00 | 128000.00 | 134666.67 | 162333.33 | 136666.67 | 162333.33 | 119333.33 | 128000.00 |
| | Log (N0/N) | 0.03 | | 0.06 | | -0.02 | | 0.05 | | 0.07 | | 0.03 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| 360 | STDEV | 0.0 | | | | 0.0 | | | | 0.0 | | | |

Table C 12: Effect of Gallic acid on *B. cepacia* growth

| | | Gallic acid 1MIC | | | | Gallic acid 2MIC | | | | Gallic acid 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Replicate 1 | | Replicate 2 | | Replicate 1 | | Replicate 2 | | Replicate 1 | | Replicate 2 | |
| contact time (min) | sample | sample | control | sample | control | sample | control | sample | control | sample | control | sample | control |
| | Mean colonies | 112.33 | 111.33 | 135.00 | 138.67 | 106.00 | 111.33 | 140.67 | 138.67 | 115.67 | 111.33 | 132.00 | 138.67 |
| | stdev | 3.06 | 11.24 | 13.89 | 13.05 | 1.41 | 11.24 | 8.14 | 13.05 | 10.97 | 11.24 | 2.12 | 13.05 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 112333.33 | 111333.33 | 135000.00 | 138666.67 | 106000.00 | 111333.33 | 140666.67 | 138666.67 | 115666.67 | 111333.33 | 132000.00 | 138666.67 |
| | $Log_{10}(N_o/N)$ | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 0 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 99.67 | 104.33 | 123.67 | 128.00 | 97.33 | 104.33 | 123.50 | 128.00 | 100.67 | 104.33 | 127.67 | 128.00 |
| | stdev | 9.71 | 2.08 | 5.51 | 5.57 | 1.53 | 2.08 | 4.95 | 5.57 | 10.21 | 2.08 | 12.10 | 5.57 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 99666.67 | 104333.33 | 123666.67 | 128000.00 | 97333.33 | 104333.33 | 123500.00 | 128000.00 | 100666.67 | 104333.33 | 127666.67 | 128000.00 |
| | $Log_{10}(N_o/N)$ | 0.05 | | 0.04 | | 0.04 | | 0.06 | | 0.06 | | 0.01 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 15 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 107.33 | 123.00 | 124.00 | 133.00 | 103.33 | 123.00 | 125.00 | 133.00 | 89.00 | 123.00 | 128.67 | 133.00 |
| | stdev | 11.93 | 4.24 | 5.29 | 12.77 | 7.23 | 4.24 | 3.54 | 12.77 | 5.66 | 4.24 | 16.44 | 12.77 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 107333.33 | 123000.00 | 124000.00 | 133000.00 | 103333.33 | 123000.00 | 125000.00 | 133000.00 | 89000.00 | 123000.00 | 128666.67 | 133000.00 |
| | $Log_{10}(N_o/N)$ | 0.02 | | 0.04 | | 0.01 | | 0.05 | | 0.11 | | 0.01 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| 30 | stdev | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| | Mean colonies | 112.00 | 107.67 | 124.00 | 135.00 | 105.00 | 107.67 | 125.67 | 135.00 | 112.33 | 107.67 | 121.00 | 135.00 |
| | stdev | 3.46 | 14.01 | 7.07 | 6.08 | 3.46 | 14.01 | 1.53 | 6.08 | 16.77 | 14.01 | 3.61 | 6.08 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 112000.00 | 107666.67 | 124000.00 | 135000.00 | 105000.00 | 107666.67 | 125666.67 | 135000.00 | 112333.33 | 107666.67 | 121000.00 | 135000.00 |
| | $Log_{10}(N_o/N)$ | 0.00 | | 0.04 | | 0.00 | | 0.05 | | 0.01 | | 0.04 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 60 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 105.33 | 108.00 | 127.33 | 131.67 | 97.67 | 108.00 | 123.50 | 131.67 | 104.00 | 108.00 | 135.33 | 131.67 |
| | stdev | 10.79 | 2.83 | 8.50 | 6.66 | 9.29 | 2.83 | 6.36 | 6.66 | 14.00 | 2.83 | 4.93 | 6.66 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 105333.33 | 108000.00 | 127333.33 | 131666.67 | 97666.67 | 108000.00 | 123500.00 | 131666.67 | 104000.00 | 108000.00 | 135333.33 | 131666.67 |
| | $Log_{10}(N_o/N)$ | 0.03 | | 0.03 | | 0.04 | | 0.06 | | 0.05 | | -0.01 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 120 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 95.67 | 111.67 | 113.33 | 133.33 | 104.00 | 111.67 | 118.33 | 133.33 | 99.67 | 111.67 | 117.67 | 133.33 |
| | stdev | 11.72 | 2.52 | 18.15 | 16.04 | 9.54 | 2.52 | 3.51 | 16.04 | 12.50 | 2.52 | 4.16 | 16.04 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 95666.67 | 111666.67 | 113333.33 | 133333.33 | 104000.00 | 111666.67 | 118333.33 | 133333.33 | 99666.67 | 111666.67 | 117666.67 | 133333.33 |
| | $Log_{10}(N_o/N)$ | 0.07 | | 0.08 | | 0.01 | | 0.08 | | 0.06 | | 0.05 | |
| | Mean | 0.1 | | | | 0.0 | | | | 0.1 | | | |
| 240 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 89.00 | 112.00 | 105.00 | 127.00 | 100.50 | 112.00 | 95.67 | 127.00 | 90.67 | 112.00 | 97.33 | 127.00 |
| | stdev | 6.36 | 6.08 | 3.54 | 4.58 | 7.78 | 6.08 | 3.06 | 4.58 | 6.11 | 6.08 | 8.14 | 4.58 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 89000.00 | 112000.00 | 105000.00 | 127000.00 | 100500.00 | 112000.00 | 95666.67 | 127000.00 | 90666.67 | 112000.00 | 97333.33 | 127000.00 |
| | $Log_{10}(N_o/N)$ | 0.10 | | 0.11 | | 0.02 | | 0.17 | | 0.11 | | 0.13 | |
| | Mean | 0.1 | | | | 0.1 | | | | 0.1 | | | |
| 360 | stdev | 0.0 | | | | 0.1 | | | | 0.0 | | | |

**Appendix C5: Raw data for *A. hydrophila* disinfection**

Table C 13: Effect of Epigallocatechin gallate on *A. hydrophila* growth

| | | EGCg 1MIC | | | | EGCg 2MIC | | | | EGCg 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Replicate 1 | | Replicate 2 | | Replicate 1 | | Replicate 2 | | Replicate 1 | | Replicate 2 | |
| Contact time (min) | sample | sample | control | sample | control | sample | control | sample | control | sample | control | sample | control |
| | Mean colonies | 126.33 | 126.33 | 79.00 | 88.00 | 126.33 | 126.33 | 73.67 | 88.00 | 126.33 | 126.33 | 98.33 | 88.00 |
| | stdev | 13.58 | 13.58 | 7.21 | 5.66 | 13.58 | 13.58 | 4.62 | 5.66 | 13.58 | 13.58 | 26.58 | 5.66 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 126333.33 | 126333.33 | 79000.00 | 88000.00 | 126333.33 | 126333.33 | 73666.67 | 88000.00 | 126333.33 | 126333.33 | 98333.33 | 88000.00 |
| | $Log_{10}(N_o/N)$ | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 0 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 106.00 | 128.50 | 71.67 | 94.00 | 94.50 | 128.50 | 68.67 | 94.00 | 91.50 | 128.50 | 84.33 | 94.00 |
| | stdev | 24.04 | 14.85 | 3.51 | 2.83 | 12.02 | 14.85 | 7.77 | 2.83 | 2.12 | 14.85 | 8.50 | 2.83 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 106000.00 | 128500.00 | 71666.67 | 94000.00 | 94500.00 | 128500.00 | 68666.67 | 94000.00 | 91500.00 | 128500.00 | 84333.33 | 94000.00 |
| | $Log_{10}(N_o/N)$ | 0.08 | | 0.04 | | 0.13 | | 0.03 | | 0.14 | | 0.07 | |
| | Mean | 0.1 | | | | 0.1 | | | | 0.1 | | | |
| 15 | stdev | 0.0 | | | | 0.1 | | | | 0.1 | | | |
| | Mean colonies | 106.00 | 114.00 | 58.50 | 83.00 | 91.00 | 114.00 | 63.00 | 83.00 | 80.00 | 114.00 | 67.33 | 83.00 |
| | stdev | 4.24 | 43.84 | 3.54 | 11.31 | 9.90 | 43.84 | 14.14 | 11.31 | 2.83 | 43.84 | 6.43 | 11.31 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 106000.00 | 114000.00 | 58500.00 | 83000.00 | 91000.00 | 114000.00 | 63000.00 | 83000.00 | 80000.00 | 114000.00 | 67333.33 | 83000.00 |
| | $Log_{10}(N_o/N)$ | 0.08 | | 0.13 | | 0.14 | | 0.07 | | 0.20 | | 0.16 | |
| | Mean | 0.1 | | | | 0.1 | | | | 0.2 | | | |
| 30 | stdev | 0.0 | | | | 0.1 | | | | 0.0 | | | |
| | Mean colonies | 101.00 | 126.33 | 59.67 | 87.00 | 91.00 | 126.33 | 60.67 | 87.00 | 76.00 | 126.33 | 67.00 | 87.00 |
| | stdev | 8.49 | 15.08 | 13.61 | 22.63 | 2.83 | 15.08 | 14.47 | 22.63 | 4.24 | 15.08 | 4.24 | 22.63 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 101000.00 | 126333.33 | 59666.67 | 87000.00 | 91000.00 | 126333.33 | 60666.67 | 87000.00 | 76000.00 | 126333.33 | 67000.00 | 87000.00 |
| | $Log_{10}(N_o/N)$ | 0.10 | | 0.12 | | 0.14 | | 0.08 | | 0.22 | | 0.17 | |
| | Mean | 0.1 | | | | 0.1 | | | | 0.2 | | | |
| 60 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 92.50 | 128.00 | 58.33 | 88.00 | 89.00 | 128.00 | 65.00 | 88.00 | 77.00 | 128.00 | 54.33 | 88.00 |
| | stdev | 0.71 | 19.80 | 7.51 | 12.73 | 7.07 | 19.80 | 4.58 | 12.73 | 2.83 | 19.80 | 5.03 | 12.73 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 92500.00 | 128000.00 | 58333.33 | 88000.00 | 89000.00 | 128000.00 | 65000.00 | 88000.00 | 77000.00 | 128000.00 | 54333.33 | 88000.00 |
| | $Log_{10}(N_o/N)$ | 0.14 | | 0.13 | | 0.15 | | 0.05 | | 0.22 | | 0.26 | |
| | Mean | 0.1 | | | | 0.1 | | | | 0.2 | | | |
| 120 | stdev | 0.0 | | | | 0.1 | | | | 0.0 | | | |
| | Mean colonies | 87.50 | 120.00 | 50.00 | 102.00 | 76.50 | 120.00 | 56.50 | 102.00 | 77.50 | 120.00 | 44.00 | 102.00 |
| | stdev | 10.61 | 21.21 | 9.90 | 7.55 | 17.68 | 21.21 | 10.57 | 7.55 | 4.95 | 21.21 | 0.00 | 7.55 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 87500.00 | 120000.00 | 50000.00 | 102000.00 | 76500.00 | 120000.00 | 56500.00 | 102000.00 | 77500.00 | 120000.00 | 44000.00 | 102000.00 |
| | $Log_{10}(N_o/N)$ | 0.16 | | 0.20 | | 0.22 | | 0.12 | | 0.21 | | 0.35 | |
| | Mean | 0.2 | | | | 0.2 | | | | 0.3 | | | |
| 240 | stdev | 0.0 | | | | 0.1 | | | | 0.1 | | | |
| | Mean colonies | 92.00 | 127.00 | 51.67 | 95.33 | 63.50 | 127.00 | 41.33 | 95.33 | 59.00 | 127.00 | 40.00 | 95.33 |
| | stdev | 0.00 | 19.09 | 5.51 | 19.04 | 13.44 | 19.09 | 4.04 | 19.04 | 6.36 | 19.09 | 0.00 | 19.04 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 92000.00 | 127000.00 | 51666.67 | 95333.33 | 63500.00 | 127000.00 | 41333.33 | 95333.33 | 59000.00 | 127000.00 | 40000.00 | 95333.33 |
| | $Log_{10}(N_o/N)$ | 0.14 | | 0.18 | | 0.30 | | 0.25 | | 0.33 | | 0.39 | |
| | Mean | 0.2 | | | | 0.3 | | | | 0.4 | | | |
| 360 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |

Table C 14: Effect of Gallic acid on *A. hydrophila* growth

| | | Gallic acid 1MIC | | | | Gallic acid 2MIC | | | | Gallic acid 4MIC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Replicate 1 | | Replicate 2 | | Replicate 1 | | Replicate 2 | | Replicate 1 | | Replicate 2 | |
| Contact time (min) | sample | sample | control | sample | control | sample | control | sample | control | sample | control | sample | control |
| | Mean colonies | 123.67 | 148.00 | 97.00 | 94.33 | 143.00 | 148.00 | 96.33 | 94.33 | 141.00 | 148.00 | 89.50 | 94.33 |
| | stdev | 11.72 | 11.14 | 2.65 | 1.53 | 2.12 | 11.14 | 5.13 | 1.53 | 2.83 | 11.14 | 2.12 | 1.53 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 123666.67 | 148000.00 | 97000.00 | 94333.33 | 143000.00 | 148000.00 | 96333.33 | 94333.33 | 141000.00 | 148000.00 | 89500.00 | 94333.33 |
| | $Log_{10}(N_o/N)$ | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 0 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 139.67 | 119.33 | 81.67 | 92.67 | 135.00 | 119.33 | 87.50 | 92.67 | 128.67 | 119.33 | 88.50 | 92.67 |
| | stdev | 10.02 | 18.82 | 12.58 | 2.89 | 9.54 | 18.82 | 3.54 | 2.89 | 12.34 | 18.82 | 3.54 | 2.89 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 139666.67 | 119333.33 | 81666.67 | 92666.67 | 135000.00 | 119333.33 | 87500.00 | 92666.67 | 128666.67 | 119333.33 | 88500.00 | 92666.67 |
| | $Log_{10}(N_o/N)$ | -0.05 | | 0.07 | | 0.03 | | 0.04 | | 0.04 | | 0.00 | |
| | Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| 15 | stdev | 0.1 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 138.00 | 148.00 | 112.50 | 97.00 | 150.00 | 148.00 | 88.50 | 97.00 | 134.00 | 148.00 | 92.67 | 97.00 |
| | stdev | 9.19 | 39.60 | 10.61 | 4.95 | 14.14 | 39.60 | 10.61 | 4.95 | 21.21 | 39.60 | 13.20 | 4.95 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 138000.00 | 148000.00 | 112500.00 | 97000.00 | 150000.00 | 148000.00 | 88500.00 | 97000.00 | 134000.00 | 148000.00 | 92666.67 | 97000.00 |
| | $Log_{10}(N_o/N)$ | -0.05 | | -0.06 | | -0.02 | | 0.04 | | 0.02 | | -0.02 | |
| | Mean | -0.1 | | | | 0.0 | | | | 0.0 | | | |
| 30 | stdev | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| | Mean colonies | 136.67 | 138.50 | 116.67 | 101.00 | 126.00 | 138.50 | 85.33 | 101.00 | 118.67 | 138.50 | 94.67 | 101.00 |
| | stdev | 10.21 | 21.92 | 9.29 | 12.73 | 24.43 | 21.92 | 2.08 | 12.73 | 17.56 | 21.92 | 10.41 | 12.73 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 136666.67 | 138500.00 | 116666.67 | 101000.00 | 126000.00 | 138500.00 | 85333.33 | 101000.00 | 118666.67 | 138500.00 | 94666.67 | 101000.00 |
| | $Log_{10}(N_o/N)$ | -0.04 | | -0.08 | | 0.05 | | 0.05 | | 0.07 | | -0.02 | |
| | Mean | -0.1 | | | | 0.1 | | | | 0.0 | | | |
| 60 | stdev | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| | Mean colonies | 119.33 | 142.67 | 126.33 | 101.33 | 113.00 | 142.67 | 75.33 | 101.33 | 98.00 | 142.67 | 76.33 | 101.33 |
| | stdev | 29.19 | 20.13 | 8.08 | 9.02 | 13.89 | 20.13 | 6.03 | 9.02 | 16.09 | 20.13 | 2.08 | 9.02 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 119333.33 | 142666.67 | 126333.33 | 101333.33 | 113000.00 | 142666.67 | 75333.33 | 101333.33 | 98000.00 | 142666.67 | 76333.33 | 101333.33 |
| | $Log_{10}(N_o/N)$ | 0.02 | | -0.11 | | 0.10 | | 0.11 | | 0.16 | | 0.07 | |
| | Mean | 0.0 | | | | 0.1 | | | | 0.1 | | | |
| 120 | stdev | 0.1 | | | | 0.0 | | | | 0.1 | | | |
| | Mean colonies | 101.67 | 137.00 | 114.00 | 136.00 | 110.00 | 137.00 | 57.00 | 136.00 | 80.00 | 137.00 | 56.33 | 136.00 |
| | stdev | 10.21 | 16.97 | 1.41 | 9.54 | 16.97 | 16.97 | 8.49 | 9.54 | 15.62 | 16.97 | 9.61 | 9.54 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 101666.67 | 137000.00 | 114000.00 | 136000.00 | 110000.00 | 137000.00 | 57000.00 | 136000.00 | 80000.00 | 137000.00 | 56333.33 | 136000.00 |
| | $Log_{10}(N_o/N)$ | 0.09 | | -0.07 | | 0.11 | | 0.23 | | 0.25 | | 0.20 | |
| | Mean | 0.0 | | | | 0.2 | | | | 0.2 | | | |
| 240 | stdev | 0.1 | | | | 0.1 | | | | 0.0 | | | |
| | Mean colonies | 109.33 | 152.00 | 102.67 | 117.33 | 93.00 | 152.00 | 54.67 | 117.33 | 84.00 | 152.00 | 51.00 | 117.33 |
| | stdev | 10.02 | 24.06 | 16.26 | 8.50 | 18.08 | 24.06 | 12.06 | 8.50 | 2.00 | 24.06 | 5.66 | 8.50 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 | 10^-4 |
| | cfu/ml | 109333.33 | 152000.00 | 102666.67 | 117333.33 | 93000.00 | 152000.00 | 54666.67 | 117333.33 | 84000.00 | 152000.00 | 51000.00 | 117333.33 |
| | $Log_{10}(N_o/N)$ | 0.05 | | -0.02 | | 0.19 | | 0.25 | | 0.22 | | 0.24 | |
| | Mean | 0.0 | | | | 0.2 | | | | 0.2 | | | |
| 360 | stdev | 0.1 | | | | 0.0 | | | | 0.0 | | | |

**Appendix D: Supplementary information for synergy experiment**

**Appendix D1: Raw data for compounds combination to *E. coli***

Table D 1: Combination of GA and EGCg to *E. coli* NCTC12241

| MIC GA in combination(µg/ml) | MIC EGCg in combination (µg/ml) | FIC GA | FIC EGCg | ∑FIC |
|---|---|---|---|---|
| 1.0 | 250.0 | 0.0 | 1.0 | 1.0 |
| 1.0 | 125.0 | 1.0 | 0.5 | 1.5 |
| 62.5 | 62.5 | 1.0 | 0.3 | 1.3 |
| 125.0 | 31.3 | 1.0 | 0.1 | 1.1 |
| 125.0 | 16.0 | 1.0 | 0.1 | 1.1 |
| 125.0 | 8.0 | 1.0 | 0.0 | 1.0 |

Table D 2: Combination of GA and EC to *E.coli* NCTC12241

| MIC GA in combination (µg/ml) | MIC EC in combination (µg/ml) | FIC GA | FIC EC | ∑FIC |
|---|---|---|---|---|
| 0.5 | 2000.0 | 0.0 | 1.5 | 1.5 |
| 125.0 | 1000.0 | 1.0 | 0.8 | 2.8 |
| 125.0 | 500.0 | 1.0 | 0.4 | 2.4 |
| 125.0 | 250.0 | 1.0 | 0.2 | 2.2 |
| 125.0 | 126.0 | 1.0 | 0.1 | 2.1 |
| 125.0 | 62.6 | 1.0 | 0.0 | 2.0 |

Table D 3: Combination of GA and C to *E. coli* NCTC12241

| MIC GA in combination (µg/ml) | MIC C in combination (µg/ml) | FIC GA | FIC C | ∑FIC |
|---|---|---|---|---|
| 0.5 | 1750.0 | 0.0 | 1.0 | 1.0 |
| 125.0 | 875.0 | 1.0 | 0.5 | 1.5 |
| 125.0 | 437.5 | 1.0 | 0.3 | 1.3 |
| 125.0 | 218.8 | 1.0 | 0.1 | 1.1 |
| 125.0 | 109.4 | 1.0 | 0.1 | 1.1 |
| 125.0 | 54.7 | 1.0 | 0.0 | 1.0 |

**Appendix D2: Raw data for S.aureus synergy-disinfection study**

Table D 4: Combination of Epigallocatechin gallate and gallic acid to *S. aureus* growth

| | | GA-EGCg combination 1 | | | | GA-EGCg combination 2 | | | | GA-EGCg combination 3 | | | | GA-EGCg combination 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| contact time (min) | sample | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | Mean colonies | 131.33 | 151.00 | 151.00 | 145.00 | 73.33 | 77.33 | 88.67 | 74.67 | 183.00 | 147.00 | 159.67 | 154.33 | 152.33 | 155.00 | 154.00 | 170.00 |
| | stdev | 11.02 | 28.51 | 29.14 | 24.76 | 25.70 | 10.97 | 10.26 | 4.04 | 20.66 | 11.53 | 19.55 | 23.80 | 1.53 | 7.94 | 7.94 | 58.66 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1313333.33 | 1510000.00 | 1510000.00 | 1450000.00 | 733333.33 | 773333.33 | 886666.67 | 746666.67 | 1830000.00 | 1470000.00 | 1596666.67 | 1543333.33 | 1523333.33 | 1550000.00 | 1540000.00 | 1700000.00 |
| | $log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Mean | | 0.0 | | | | 0.0 | | | | 0.0 | | | | 0.0 | | |
| 0 | STDEV | | 0.0 | | | | 0.0 | | | | 0.0 | | | | 0.0 | | |
| | Mean colonies | 115.00 | 79.33 | 88.33 | 150.33 | 26.67 | 23.67 | 92.33 | 96.00 | 162.67 | 161.33 | 134.33 | 139.67 | 17.00 | 8.67 | 14.33 | 134.00 |
| | stdev | 5.57 | 3.21 | 3.79 | 22.50 | 4.73 | 14.15 | 16.17 | 20.07 | 18.88 | 34.96 | 23.97 | 23.44 | 6.00 | 2.52 | 0.58 | 20.88 |
| | dilution factor | 10^-4 | 10^-4 | 10^-4 | 10^-5 | 10^-4 | 10^-4 | 10^-4 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-4 | 10^-4 | 10^-4 | 10^-5 |
| | cfu/ml | 115000.00 | 79333.33 | 88333.33 | 1503333.33 | 26666.67 | 23666.67 | 92333.33 | 960000.00 | 1626666.67 | 1613333.33 | 1343333.33 | 1396666.67 | 17000.00 | 8666.67 | 14333.33 | 1340000.00 |
| | $log_{10}(N_o/N)$ | 1.06 | 1.28 | 1.23 | -0.02 | 1.44 | 1.51 | 0.98 | -0.11 | 0.05 | -0.04 | 0.08 | 0.04 | 1.95 | 2.25 | 2.03 | 0.10 |
| | Mean | | 1.2 | | | | 1.3 | | | | 0.0 | | | | 2.1 | | |
| 15 | STDEV | | 0.1 | | | | 0.3 | | | | 0.1 | | | | 0.2 | | |
| | Mean colonies | 17.50 | 7.67 | 9.00 | 125.00 | 12.50 | 20.67 | 20.00 | 125.00 | 127.00 | 149.33 | 118.00 | 140.33 | 16.50 | 22.33 | 23.00 | 123.67 |
| | stdev | 8.74 | 4.93 | 1.73 | 19.92 | 2.65 | 1.15 | 2.00 | 19.92 | 20.82 | 20.53 | 7.21 | 14.43 | 0.58 | 2.52 | 1.73 | 14.47 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-5 | 10^-3 | 10^-3 | 10^-3 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-2 | 10^-2 | 10^-2 | 10^-5 |
| | cfu/ml | 1750.00 | 766.67 | 900.00 | 1250000.00 | 1250.00 | 2066.67 | 2000.00 | 1250000.00 | 1270000.00 | 1493333.33 | 1180000.00 | 1403333.33 | 165.00 | 223.33 | 230.00 | 1236666.67 |
| | $log_{10}(N_o/N)$ | 2.88 | 3.29 | 3.22 | 0.06 | 2.77 | 2.57 | 2.65 | -0.22 | 0.16 | -0.01 | 0.13 | 0.04 | 3.97 | 3.84 | 3.83 | |
| | Mean | | 3.1 | | | | 2.7 | | | | 0.1 | | | | 3.9 | | |
| 30 | STDEV | | 0.2 | | | | 0.1 | | | | 0.1 | | | | 0.1 | | |
| | Mean colonies | 21.67 | 16.00 | 28.67 | 93.50 | 1.33 | 1.00 | 5.67 | 93.50 | 112.00 | 114.67 | 112.00 | 132.00 | 3.00 | 3.67 | 7.33 | 170.50 |
| | stdev | 7.64 | 2.00 | 4.93 | 4.36 | 1.53 | 1.00 | 5.51 | 4.36 | 12.12 | 1.53 | 2.65 | 5.29 | 1.00 | 2.08 | 0.58 | 20.26 |
| | dilution factor | 10^-1 | 10^-1 | 10^-1 | 10^-5 | 10^-2 | 10^-2 | 10^-2 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-2 | 10^-2 | 10^-2 | 10^-5 |
| | cfu/ml | 21.67 | 16.00 | 28.67 | 935000.00 | 13.33 | 10.00 | 56.67 | 935000.00 | 1120000.00 | 1146666.67 | 1120000.00 | 1320000.00 | 30.00 | 36.67 | 73.33 | 1705000.00 |
| | $log_{10}(N_o/N)$ | 4.78 | 4.97 | 4.72 | 0.19 | 4.74 | 4.89 | 4.19 | -0.10 | 0.21 | 0.11 | 0.15 | 0.07 | 4.71 | 4.63 | 4.32 | 0.00 |
| | Mean | | 4.8 | | | | 4.6 | | | | 0.2 | | | | 4.6 | | |
| 60 | STDEV | | 0.1 | | | | 0.4 | | | | 0.1 | | | | 0.2 | | |
| | Mean colonies | 4.67 | 3.00 | 5.33 | 146.50 | 8.00 | 3.67 | 35.33 | 146.50 | 102.33 | 97.00 | 100.33 | 147.50 | 26.00 | 15.00 | 22.00 | 148.00 |
| | stdev | 3.06 | 1.00 | 1.53 | 30.27 | 8.00 | 2.52 | 4.73 | 30.27 | 13.65 | 6.56 | 6.51 | 2.89 | 4.36 | 2.00 | 1.00 | 17.90 |
| | dilution factor | 10^-1 | 10^-1 | 10^-1 | 10^-5 | 10^-1 | 10^-1 | 10^-1 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-1 | 10^-1 | 10^-1 | 10^-5 |
| | cfu/ml | 4.67 | 3.00 | 5.33 | 1465000.00 | 8.00 | 3.67 | 35.33 | 1465000.00 | 1023333.33 | 970000.00 | 1003333.33 | 1475000.00 | 26.00 | 15.00 | 22.00 | 1480000.00 |
| | $log_{10}(N_o/N)$ | 5.45 | 5.70 | 5.45 | 0.00 | 4.96 | 5.32 | 4.40 | -0.29 | 0.25 | 0.18 | 0.20 | 0.02 | 4.77 | 5.01 | 4.85 | 0.06 |
| | Mean | | 5.5 | | | | 4.9 | | | | 0.2 | | | | 4.9 | | |
| 120 | STDEV | | 0.1 | | | | 0.5 | | | | 0.0 | | | | 0.1 | | |
| | Mean colonies | 61.00 | 92.67 | 127.67 | 117.00 | 119.33 | 81.33 | 99.00 | 90.00 | 92.33 | 88.00 | 99.00 | 141.50 | 255.33 | 143.33 | 222.00 | 90.00 |
| | stdev | 1.00 | 10.50 | 10.12 | 14.73 | 2.31 | 19.60 | 12.53 | 19.29 | 9.81 | 9.54 | 8.19 | 10.50 | 13.05 | 26.27 | 23.00 | 19.29 |
| | dilution factor | no dilution | no dilution | no dilution | 10^-5 | no dilution | no dilution | no dilution | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | no dilution | no dilution | no dilution | 10^-5 |
| | cfu/ml | 6.10 | 9.27 | 12.77 | 1170000.00 | 11.93 | 8.13 | 9.90 | 900000.00 | 923333.33 | 880000.00 | 990000.00 | 1415000.00 | 25.53 | 14.33 | 22.20 | 900000.00 |
| | $log_{10}(N_o/N)$ | 5.33 | 5.21 | 5.07 | 0.09 | 4.79 | 4.98 | 4.95 | -0.08 | 0.30 | 0.22 | 0.21 | 0.04 | 4.78 | 5.03 | 4.84 | 0.28 |
| | Mean | | 5.2 | | | | 4.9 | | | | 0.2 | | | | 4.9 | | |
| 240 | STDEV | | 0.1 | | | | 0.1 | | | | 0.0 | | | | 0.1 | | |
| | Mean colonies | 43.33 | 62.00 | 81.00 | 131.50 | 97.33 | 77.33 | 68.67 | 107.00 | 96.33 | 95.67 | 101.33 | 142.50 | 158.00 | 177.33 | 196.00 | 201.50 |
| | stdev | 10.21 | 9.85 | 6.00 | 16.50 | 12.66 | 12.50 | 16.77 | 16.17 | 15.18 | 5.13 | 7.57 | 10.41 | 37.03 | 13.50 | 14.11 | 45.72 |
| | dilution factor | no dilution | no dilution | no dilution | 10^-5 | no dilution | no dilution | no dilution | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | no dilution | no dilution | no dilution | 10^-5 |
| | cfu/ml | 4.33 | 6.20 | 8.10 | 1315000.00 | 9.73 | 7.73 | 6.87 | 1070000.00 | 963333.33 | 956666.67 | 1013333.33 | 1425000.00 | 15.80 | 17.73 | 19.60 | 2015000.00 |
| | $log_{10}(N_o/N)$ | 5.48 | 5.39 | 5.27 | 0.04 | 4.88 | 5.00 | 5.11 | -0.16 | 0.28 | 0.19 | 0.20 | 0.03 | 4.98 | 4.94 | 4.90 | -0.07 |
| | Mean | | 5.4 | | | | 5.0 | | | | 0.2 | | | | 4.9 | | |
| 360 | STDEV | | 0.1 | | | | 0.1 | | | | 0.1 | | | | 0.0 | | |

Table D 5: Combination of Epicatechin and gallic acid to *S. aureus* growth

| | | GA-EC combination 1 | | | | GA-EC combination 2 | | | | GA-EC-combination 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| contact time (min) | sample | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | Mean colonies | 176.67 | 160.00 | 149.00 | 155.67 | 115.33 | 135.00 | 140.00 | 137.33 | 140.33 | 165.00 | 152.00 | 167.33 |
| | stdev | 23.46 | 24.43 | 29.14 | 27.93 | 14.01 | 19.05 | 11.14 | 27.43 | 16.26 | 21.17 | 21.17 | 19.55 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1766666.67 | 1600000.00 | 1490000.00 | 1556666.67 | 1153333.33 | 1350000.00 | 1400000.00 | 1373333.33 | 1403333.33 | 1650000.00 | 1520000.00 | 1673333.33 |
| | $Log_{10}$ ($N_o$/N) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Mean | | 0.0 | | | | 0.0 | | | | 0.0 | | |
| 0 | STDEV | | 0.0 | | | | 0.0 | | | | 0.0 | | |
| | Mean colonies | 158.00 | 145.33 | 135.33 | 155.67 | 130.00 | 112.67 | 115.67 | 147.00 | 131.00 | 156.67 | 147.67 | 167.33 |
| | stdev | 12.12 | 12.66 | 8.08 | 27.93 | 26.06 | 8.02 | 27.32 | 17.52 | 25.16 | 29.74 | 37.54 | 19.55 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1580000.00 | 1453333.33 | 1353333.33 | 1556666.67 | 1300000.00 | 1126666.67 | 1156666.67 | 1470000.00 | 1310000.00 | 1566666.67 | 1476666.67 | 1673333.33 |
| | $Log_{10}$ ($N_o$/N) | 0.05 | 0.04 | 0.04 | 0.00 | -0.05 | 0.08 | 0.08 | -0.03 | 0.03 | 0.02 | 0.01 | 0.00 |
| | Mean | | 0.0 | | | | 0.0 | | | | 0.0 | | |
| 15 | STDEV | | 0.0 | | | | 0.1 | | | | 0.0 | | |
| | Mean colonies | 129.50 | 159.00 | 123.00 | 155.67 | 102.50 | 102.33 | 107.33 | 147.00 | 138.50 | 132.00 | 136.33 | 167.33 |
| | stdev | 1.00 | 16.00 | 16.52 | 27.93 | 10.97 | 16.44 | 10.21 | 17.52 | 28.75 | 19.16 | 11.06 | 19.55 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1295000.00 | 1590000.00 | 1230000.00 | 1556666.67 | 1025000.00 | 1023333.33 | 1073333.33 | 1470000.00 | 1385000.00 | 1320000.00 | 1363333.33 | 1673333.33 |
| | $Log_{10}$ ($N_o$/N) | 0.13 | 0.00 | 0.08 | 0.00 | 0.05 | 0.12 | 0.12 | -0.03 | 0.01 | 0.10 | 0.05 | 0.00 |
| | Mean | | 0.1 | | | | 0.1 | | | | 0.0 | | |
| 30 | STDEV | | 0.1 | | | | 0.0 | | | | 0.0 | | |
| | Mean colonies | 127.00 | 132.67 | 136.67 | 151.50 | 100.33 | 96.33 | 99.67 | 147.50 | 128.00 | 111.00 | 133.33 | 175.50 |
| | stdev | 2.65 | 15.89 | 2.08 | 15.13 | 17.62 | 4.51 | 4.73 | 17.52 | 0.00 | 22.61 | 2.31 | 17.35 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1270000.00 | 1326666.67 | 1366666.67 | 1515000.00 | 1003333.33 | 963333.33 | 996666.67 | 1475000.00 | 1280000.00 | 1110000.00 | 1333333.33 | 1755000.00 |
| | $Log_{10}$ ($N_o$/N) | 0.14 | 0.08 | 0.04 | 0.01 | 0.06 | 0.15 | 0.15 | -0.03 | 0.04 | 0.17 | 0.06 | -0.02 |
| | Mean | | 0.1 | | | | 0.1 | | | | 0.1 | | |
| 60 | STDEV | | 0.1 | | | | 0.0 | | | | 0.1 | | |
| | Mean colonies | 131.33 | 127.33 | 130.00 | 152.00 | 101.33 | 84.00 | 95.33 | 147.50 | 114.33 | 119.67 | 108.00 | 175.50 |
| | stdev | 29.02 | 20.60 | 7.55 | 23.07 | 7.37 | 2.65 | 16.44 | 4.04 | 18.45 | 10.21 | 1.00 | 17.35 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1313333.33 | 1273333.33 | 1300000.00 | 1520000.00 | 1013333.33 | 840000.00 | 953333.33 | 1475000.00 | 1143333.33 | 1196666.67 | 1080000.00 | 1755000.00 |
| | $Log_{10}$ ($N_o$/N) | 0.13 | 0.10 | 0.06 | 0.01 | 0.06 | 0.21 | 0.17 | -0.03 | 0.09 | 0.14 | 0.15 | -0.02 |
| | Mean | | 0.1 | | | | 0.1 | | | | 0.1 | | |
| 120 | STDEV | | 0.0 | | | | 0.1 | | | | 0.0 | | |
| | Mean colonies | 132.67 | 138.33 | 121.67 | 148.50 | 107.33 | 85.67 | 74.67 | 147.50 | 140.00 | 137.67 | 126.67 | 144.50 |
| | stdev | 28.45 | 4.73 | 1.53 | 5.51 | 18.90 | 4.93 | 7.57 | 4.04 | 8.66 | 11.06 | 13.61 | 20.21 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1326666.67 | 1383333.33 | 1216666.67 | 1485000.00 | 1073333.33 | 856666.67 | 746666.67 | 1475000.00 | 1400000.00 | 1376666.67 | 1266666.67 | 1445000.00 |
| | $Log_{10}$ ($N_o$/N) | 0.12 | 0.06 | 0.09 | 0.02 | 0.03 | 0.20 | 0.27 | -0.03 | 0.00 | 0.08 | 0.08 | 0.06 |
| | Mean | | 0.1 | | | | 0.2 | | | | 0.1 | | |
| 240 | STDEV | | 0.0 | | | | 0.1 | | | | 0.0 | | |
| | Mean colonies | 113.67 | 109.33 | 106.00 | 151.00 | 51.00 | 59.00 | 66.33 | 145.67 | 128.33 | 128.00 | 127.33 | 159.00 |
| | stdev | 21.36 | 3.51 | 7.00 | 35.00 | 7.55 | 10.39 | 3.06 | 4.04 | 19.50 | 1.00 | 4.93 | 6.08 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1136666.67 | 1093333.33 | 1060000.00 | 1510000.00 | 510000.00 | 590000.00 | 663333.33 | 1456666.67 | 1283333.33 | 1280000.00 | 1273333.33 | 1590000.00 |
| | $Log_{10}$ ($N_o$/N) | 0.19 | 0.17 | 0.15 | 0.01 | 0.35 | 0.36 | 0.32 | -0.03 | 0.04 | 0.11 | 0.08 | 0.02 |
| | Mean | | 0.2 | | | | 0.3 | | | | 0.1 | | |
| 360 | STDEV | | 0.0 | | | | 0.0 | | | | 0.0 | | |

Table D 6: Combination of catechin and gallic acid to *S. aureus* growth

| | | GA-C combination 1 | | | | GA-C combination 2 | | | | GA-C combinaton 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| contact time (min) | sample | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | Mean colonies | 132.33 | 112.00 | 131.67 | 135.00 | 161.00 | 184.33 | 198.67 | 191.67 | 143.00 | 146.67 | 147.33 | 138.00 |
| | stdev | 7.09 | 8.02 | 18.61 | 16.37 | 13.08 | 11.59 | 34.20 | 34.53 | 3.61 | 0.58 | 6.03 | 22.87 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1323333.33 | 1120000.00 | 1316666.67 | 1350000.00 | 1610000.00 | 1843333.33 | 1986666.67 | 1916666.67 | 1430000.00 | 1466666.67 | 1473333.33 | 1380000.00 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean colonies | 115.33 | 120.33 | 127.67 | 131.00 | 152.67 | 155.00 | 169.00 | 161.00 | 165.67 | 146.00 | 139.00 | 140.67 |
| | stdev | 6.81 | 5.69 | 15.53 | 20.22 | 34.15 | 19.47 | 25.98 | 26.96 | 20.60 | 15.00 | 16.46 | 9.24 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1153333.33 | 1203333.33 | 1276666.67 | 1310000.00 | 1526666.67 | 1550000.00 | 1690000.00 | 1610000.00 | 1656666.67 | 1460000.00 | 1390000.00 | 1406666.67 |
| | $Log_{10}(N_o/N)$ | 0.06 | -0.03 | 0.01 | 0.01 | 0.02 | 0.08 | 0.07 | 0.08 | -0.06 | 0.00 | 0.03 | -0.01 |
| | Mean | | | 0.0 | | | | 0.1 | | | | 0.0 | |
| 15 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean colonies | 111.00 | 115.67 | 127.33 | 127.33 | 155.00 | 135.00 | 166.33 | 158.00 | 144.50 | 105.67 | 128.67 | 139.33 |
| | stdev | 10.12 | 9.29 | 24.03 | 23.03 | 12.90 | 6.24 | 31.21 | 2.00 | 12.50 | 13.65 | 17.47 | 1.15 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1110000.00 | 1156666.67 | 1273333.33 | 1273333.33 | 1550000.00 | 1350000.00 | 1663333.33 | 1580000.00 | 1445000.00 | 1056666.67 | 1286666.67 | 1393333.33 |
| | $Log_{10}(N_o/N)$ | 0.08 | -0.01 | 0.01 | 0.03 | 0.02 | 0.14 | 0.08 | 0.08 | 0.00 | 0.14 | 0.06 | 0.00 |
| | Mean | | | 0.0 | | | | 0.1 | | | | 0.1 | |
| 30 | STDEV | | | 0.0 | | | | 0.1 | | | | 0.1 | |
| | Mean colonies | 114.67 | 117.33 | 119.00 | 128.00 | 130.00 | 135.00 | 133.00 | 164.00 | 107.33 | 110.00 | 118.67 | 139.00 |
| | stdev | 22.14 | 13.05 | 12.77 | 5.57 | 13.75 | 6.24 | 1.00 | 7.57 | 11.68 | 13.11 | 7.51 | 4.04 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1146666.67 | 1173333.33 | 1190000.00 | 1280000.00 | 1300000.00 | 1350000.00 | 1330000.00 | 1640000.00 | 1073333.33 | 1100000.00 | 1186666.67 | 1390000.00 |
| | $Log_{10}(N_o/N)$ | 0.06 | -0.02 | 0.04 | 0.02 | 0.09 | 0.14 | 0.17 | 0.07 | 0.12 | 0.12 | 0.09 | 0.00 |
| | Mean | | | 0.0 | | | | 0.1 | | | | 0.1 | |
| 60 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean colonies | 117.00 | 108.67 | 117.67 | 126.50 | 134.67 | 117.33 | 132.00 | 164.00 | 113.33 | 99.67 | 121.00 | 137.50 |
| | stdev | 18.52 | 9.50 | 13.65 | 7.23 | 4.16 | 23.69 | 3.61 | 12.86 | 21.50 | 10.79 | 37.03 | 2.65 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1170000.00 | 1086666.67 | 1176666.67 | 1265000.00 | 1346666.67 | 1173333.33 | 1320000.00 | 1640000.00 | 1133333.33 | 996666.67 | 1210000.00 | 1375000.00 |
| | $Log_{10}(N_o/N)$ | 0.05 | 0.01 | 0.05 | 0.03 | 0.08 | 0.20 | 0.18 | 0.07 | 0.10 | 0.17 | 0.09 | 0.00 |
| | Mean | | | 0.0 | | | | 0.2 | | | | 0.1 | |
| 120 | STDEV | | | 0.0 | | | | 0.1 | | | | 0.0 | |
| | Mean colonies | 103.00 | 104.33 | 109.00 | 126.50 | 100.00 | 103.33 | 106.33 | 155.00 | 100.33 | 110.00 | 102.33 | 142.00 |
| | stdev | 4.58 | 12.06 | 7.00 | 10.69 | 5.00 | 2.52 | 5.51 | 1.73 | 10.41 | 3.61 | 10.69 | 19.73 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1030000.00 | 1043333.33 | 1090000.00 | 1265000.00 | 1000000.00 | 1033333.33 | 1063333.33 | 1550000.00 | 1003333.33 | 1100000.00 | 1023333.33 | 1420000.00 |
| | $Log_{10}(N_o/N)$ | 0.11 | 0.03 | 0.08 | 0.03 | 0.21 | 0.25 | 0.27 | 0.09 | 0.15 | 0.12 | 0.16 | -0.01 |
| | Mean | | | 0.1 | | | | 0.2 | | | | 0.1 | |
| 240 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean colonies | 101.33 | 101.67 | 118.00 | 123.67 | 111.33 | 107.00 | 90.00 | 150.67 | 112.67 | 113.67 | 101.67 | 138.67 |
| | stdev | 13.50 | 10.60 | 11.53 | 12.74 | 4.62 | 5.57 | 7.21 | 1.15 | 16.86 | 24.58 | 9.50 | 7.64 |
| | dilution factor | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 | 10^-5 |
| | cfu/ml | 1013333.33 | 1016666.67 | 1180000.00 | 1236666.67 | 1113333.33 | 1070000.00 | 900000.00 | 1506666.67 | 1126666.67 | 1136666.67 | 1016666.67 | 1386666.67 |
| | $Log_{10}(N_o/N)$ | 0.12 | 0.04 | 0.05 | 0.04 | 0.16 | 0.24 | 0.34 | 0.10 | 0.10 | 0.11 | 0.16 | 0.00 |
| | Mean | | | 0.1 | | | | 0.2 | | | | 0.1 | |
| 360 | STDEV | | | 0.0 | | | | 0.1 | | | | 0.0 | |

## Appendix E: Supplementary information for MS2 inactivation

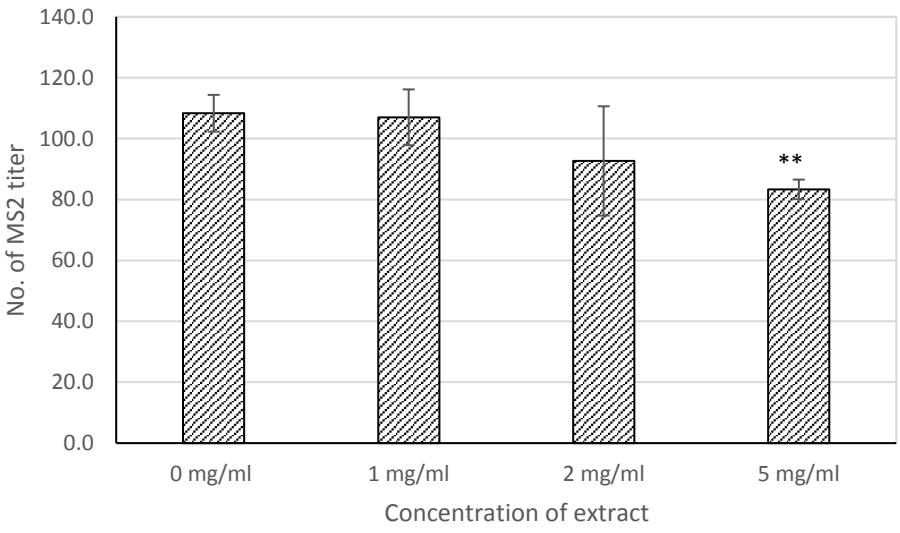
### Appendix E1: Quality control for MS2 inactivation

Table E 1: Phage stability in PBS

| | plaque formed | | | | | | |
|---|---|---|---|---|---|---|---|
| Contact time(min) | R1 | R2 | mean plaques | dilution factor | mean pfu/ml | log pfu/ml | stdev |
| 0.00 | 86.00 | 81.00 | 83.50 | 10^-3 | 835000.00 | 5.9 | 0.0 |
| 10.00 | 90.00 | 95.00 | 92.50 | 10^-3 | 925000.00 | 6.0 | 0.0 |
| 20.00 | 108.00 | 94.00 | 101.00 | 10^-3 | 1010000.00 | 6.0 | 0.0 |
| 30.00 | 108.00 | 110.00 | 109.00 | 10^-3 | 1090000.00 | 6.0 | 0.0 |
| 40.00 | 109.00 | 97.00 | 103.00 | 10^-3 | 1030000.00 | 6.0 | 0.0 |
| 50.00 | 86.00 | 106.00 | 96.00 | 10^-3 | 960000.00 | 6.0 | 0.1 |
| 60.00 | 107.00 | 93.00 | 100.00 | 10^-3 | 1000000.00 | 6.0 | 0.0 |

### Appendix E2: Preliminary testing for MS2 tolerance to DMSO

This preliminary study was conducted to determine the number of MS2 titer remaining after DMSO addition, in order to understand whether the DMSO used during the extracts preparation may reduce the number of MS2 during the disinfection. The number of MS2 titer was measured after 10 minutes of DMSO addition.




** $p<0.05$, the number of titer statistically reduced compared to the untreated control (0 mg/ml)

## Appendix E3: Raw data for MS2 inactivation

Table E 2: Effect of banana extracts to MS2 titer

| contact time (min) | sample | FD3hr 1mg/ml | | | | FD3hr 2mg/ml | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | Mean plaques | 65.00 | 61.67 | 64.00 | 67.00 | 70.00 | 63.67 | 70.33 | 68.67 |
| | stdev | 13.89 | 2.08 | 9.64 | 9.85 | 3.00 | 2.08 | 6.66 | 10.50 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 650000.00 | 616666.67 | 640000.00 | 670000.00 | 700000.00 | 636666.67 | 703333.33 | 686666.67 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Mean | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 62.33 | 68.33 | 71.67 | 80.33 | 66.00 | 58.00 | 64.33 | 64.67 |
| | stdev | 6.66 | 8.96 | 12.10 | 13.50 | 6.24 | 3.00 | 4.62 | 4.93 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 623333.33 | 683333.33 | 716666.67 | 803333.33 | 660000.00 | 580000.00 | 643333.33 | 646666.67 |
| | $Log_{10}(N_o/N)$ | 0.02 | -0.04 | -0.05 | -0.08 | 0.03 | 0.04 | 0.04 | 0.03 |
| | Mean | | | 0.0 | | | | 0.0 | |
| 10 | STDEV | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 55.67 | 57.67 | 52.00 | 74.33 | 67.33 | 65.33 | 59.67 | 60.33 |
| | stdev | 6.51 | 2.08 | 18.68 | 22.05 | 18.50 | 5.69 | 4.51 | 6.51 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 556666.67 | 576666.67 | 520000.00 | 743333.33 | 673333.33 | 653333.33 | 596666.67 | 603333.33 |
| | $Log_{10}(N_o/N)$ | 0.07 | 0.03 | 0.09 | -0.05 | 0.02 | -0.01 | 0.07 | 0.06 |
| | Mean | | | 0.1 | | | | 0.0 | |
| 20 | STDEV | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 61.50 | 61.33 | 59.33 | 70.00 | 52.00 | 68.00 | 66.00 | 62.50 |
| | stdev | 3.51 | 8.39 | 0.58 | 6.81 | 8.33 | 2.00 | 8.89 | 8.50 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 615000.00 | 613333.33 | 593333.33 | 700000.00 | 520000.00 | 680000.00 | 660000.00 | 625000.00 |
| | $Log_{10}(N_o/N)$ | 0.02 | 0.00 | 0.03 | -0.02 | 0.13 | -0.03 | 0.03 | 0.04 |
| | Mean | | | 0.0 | | | | 0.0 | |
| 30 | STDEV | | | 0.0 | | | | 0.1 | |
| | Mean plaques | 49.00 | 50.67 | 58.33 | 68.33 | 55.33 | 58.67 | 62.33 | 56.67 |
| | stdev | 7.94 | 5.13 | 1.53 | 2.89 | 6.66 | 12.34 | 8.02 | 9.02 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 490000.00 | 506666.67 | 583333.33 | 683333.33 | 553333.33 | 586666.67 | 623333.33 | 566666.67 |
| | $Log_{10}(N_o/N)$ | 0.12 | 0.09 | 0.04 | -0.01 | 0.10 | 0.04 | 0.05 | 0.08 |
| | Mean | | | 0.1 | | | | 0.1 | |
| 40 | STDEV | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 66.33 | 76.33 | 68.67 | 77.00 | 57.00 | 68.33 | 71.33 | 61.67 |
| | stdev | 8.02 | 8.50 | 1.53 | 5.29 | 17.69 | 10.07 | 2.52 | 8.50 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 663333.33 | 763333.33 | 686666.67 | 770000.00 | 570000.00 | 683333.33 | 713333.33 | 616666.67 |
| | $Log_{10}(N_o/N)$ | -0.01 | -0.09 | -0.03 | -0.06 | 0.09 | -0.03 | -0.01 | 0.05 |
| | Mean | | | 0.0 | | | | 0.0 | |
| 60 | STDEV | | | 0.0 | | | | 0.1 | |

Table E 3: Effect of GA to MS2 titer

| Contact time (min) | sample | GA 1mM | | | | GA 2mM | | | | GA 5mM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | Mean plaques | 97.00 | 94.33 | 92.00 | 103.67 | 73.67 | 71.33 | 71.33 | 75.33 | 81.67 | 89.33 | 64.33 | 65.33 |
| | stdev | 1.73 | 2.31 | 5.20 | 10.50 | 5.86 | 6.66 | 20.01 | 6.81 | 17.16 | 14.57 | 17.90 | 8.02 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 970000.00 | 943333.33 | 920000.00 | 1036666.67 | 736666.67 | 713333.33 | 713333.33 | 753333.33 | 816666.67 | 893333.33 | 643333.33 | 653333.33 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 86.33 | 90.00 | 82.33 | 102.33 | 60.00 | 60.33 | 74.00 | 80.67 | 91.00 | 62.67 | 56.33 | 70.33 |
| | stdev | 6.35 | 16.70 | 6.51 | 6.43 | 13.23 | 10.79 | 7.21 | 5.51 | 7.21 | 9.29 | 0.58 | 15.01 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 863333.33 | 900000.00 | 823333.33 | 1023333.33 | 600000.00 | 603333.33 | 740000.00 | 806666.67 | 910000.00 | 626666.67 | 563333.33 | 703333.33 |
| | $Log_{10}(N_o/N)$ | 0.05 | 0.02 | 0.05 | 0.01 | 0.09 | 0.07 | -0.02 | -0.03 | -0.05 | 0.15 | 0.06 | -0.03 |
| | Mean | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| 10 | STDEV | | | 0.0 | | | | 0.1 | | | | 0.1 | |
| | Mean plaques | 84.67 | 78.00 | 79.67 | 101.00 | 57.00 | 65.67 | 69.33 | 84.00 | 64.33 | 63.67 | 62.00 | 92.33 |
| | stdev | 6.66 | 5.29 | 6.66 | 7.94 | 7.21 | 9.29 | 6.43 | 3.00 | 4.51 | 11.02 | 4.00 | 4.93 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 846666.67 | 780000.00 | 796666.67 | 1010000.00 | 570000.00 | 656666.67 | 693333.33 | 840000.00 | 643333.33 | 636666.67 | 620000.00 | 923333.33 |
| | $Log_{10}(N_o/N)$ | 0.06 | 0.08 | 0.06 | 0.01 | 0.11 | 0.04 | 0.01 | -0.05 | 0.10 | 0.15 | 0.02 | -0.15 |
| | Mean | | | 0.1 | | | | 0.1 | | | | 0.1 | |
| 20 | STDEV | | | 0.0 | | | | 0.1 | | | | 0.1 | |
| | Mean plaques | 75.50 | 75.00 | 84.50 | 103.00 | 45.50 | 50.67 | 54.67 | 78.00 | 61.50 | 53.67 | 54.00 | 87.00 |
| | stdev | 3.51 | 2.00 | 2.12 | 6.43 | 24.01 | 11.68 | 9.71 | 6.56 | 7.23 | 16.29 | 16.52 | 3.79 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 755000.00 | 750000.00 | 845000.00 | 1030000.00 | 455000.00 | 506666.67 | 546666.67 | 780000.00 | 615000.00 | 536666.67 | 540000.00 | 870000.00 |
| | $Log_{10}(N_o/N)$ | 0.11 | 0.10 | 0.04 | 0.00 | 0.21 | 0.15 | 0.12 | -0.02 | 0.12 | 0.22 | 0.08 | -0.12 |
| | Mean | | | 0.1 | | | | 0.2 | | | | 0.1 | |
| 30 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| | Mean plaques | 76.33 | 80.67 | 72.67 | 106.67 | 50.00 | 46.00 | 48.33 | 77.33 | 50.67 | 55.00 | 57.67 | 77.33 |
| | stdev | 6.03 | 3.06 | 4.93 | 13.05 | 3.61 | 5.57 | 8.02 | 8.08 | 8.08 | 6.93 | 6.66 | 13.05 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 763333.33 | 806666.67 | 726666.67 | 1066666.67 | 500000.00 | 460000.00 | 483333.33 | 773333.33 | 506666.67 | 550000.00 | 576666.67 | 773333.33 |
| | $Log_{10}(N_o/N)$ | 0.10 | 0.07 | 0.10 | -0.01 | 0.17 | 0.19 | 0.17 | -0.01 | 0.21 | 0.21 | 0.05 | -0.07 |
| | Mean | | | 0.1 | | | | 0.2 | | | | 0.2 | |
| 40 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| | Mean plaques | 78.00 | 84.33 | 80.67 | 101.00 | 71.00 | 60.33 | 67.33 | 76.00 | 71.00 | 60.33 | 67.33 | 79.33 |
| | stdev | 4.58 | 8.96 | 3.79 | 9.00 | 9.54 | 6.11 | 0.58 | 5.57 | 9.54 | 6.11 | 0.58 | 8.62 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 780000.00 | 843333.33 | 806666.67 | 1010000.00 | 710000.00 | 603333.33 | 673333.33 | 760000.00 | 710000.00 | 603333.33 | 673333.33 | 793333.33 |
| | $Log_{10}(N_o/N)$ | 0.09 | 0.05 | 0.06 | 0.01 | 0.02 | 0.07 | 0.03 | 0.00 | 0.06 | 0.17 | -0.02 | -0.08 |
| | Mean | | | 0.1 | | | | 0.0 | | | | 0.1 | |
| 60 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.1 | |

Table E 4: Effect of C to MS2 titer

| | | Catechin 1mM | | | | catechin 2mM | | | | catehin 5.5mM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| contact time (min) | sample | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | mean plaques | 122.00 | 117.00 | 121.00 | 125.00 | 148.33 | 160.33 | 154.00 | 156.00 | 163.33 | 151.67 | 153.00 | 157.00 |
| | stdev | 10.82 | 4.58 | 2.65 | 3.61 | 2.08 | 1.53 | 4.00 | 4.36 | 4.93 | 25.54 | 13.08 | 11.14 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1220000.00 | 1170000.00 | 1210000.00 | 1250000.00 | 1483333.33 | 1603333.33 | 1540000.00 | 1560000.00 | 1633333.33 | 1516666.67 | 1530000.00 | 1570000.00 |
| | $\log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean plaques | 109.33 | 114.33 | 115.00 | 115.67 | 150.33 | 146.00 | 148.00 | 151.67 | 130.67 | 138.00 | 135.33 | 147.67 |
| | stdev | 17.24 | 7.02 | 4.36 | 12.10 | 4.73 | 3.61 | 6.00 | 8.50 | 4.16 | 7.94 | 5.51 | 3.79 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1093333.33 | 1143333.33 | 1150000.00 | 1156666.67 | 1503333.33 | 1460000.00 | 1480000.00 | 1516666.67 | 1306666.67 | 1380000.00 | 1353333.33 | 1476666.67 |
| | $\log_{10}(N_o/N)$ | 0.05 | 0.01 | 0.02 | 0.03 | -0.01 | 0.04 | 0.02 | 0.01 | 0.10 | 0.04 | 0.05 | 0.03 |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| 10 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean plaques | 122.67 | 111.00 | 123.00 | 120.00 | 148.67 | 152.00 | 147.00 | 161.33 | 146.00 | 140.33 | 129.67 | 151.67 |
| | stdev | 15.04 | 3.61 | 11.27 | 3.61 | 10.41 | 8.19 | 5.29 | 5.77 | 4.58 | 5.03 | 7.51 | 6.66 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1226666.67 | 1110000.00 | 1230000.00 | 1200000.00 | 1486666.67 | 1520000.00 | 1470000.00 | 1613333.33 | 1460000.00 | 1403333.33 | 1296666.67 | 1516666.67 |
| | $\log_{10}(N_o/N)$ | 0.00 | 0.02 | -0.01 | 0.02 | 0.00 | 0.02 | 0.02 | -0.01 | 0.05 | 0.03 | 0.07 | 0.02 |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| 20 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean plaques | 116.00 | 122.00 | 120.33 | 124.33 | 152.50 | 144.00 | 158.00 | 163.00 | 139.00 | 134.33 | 147.00 | 149.00 |
| | stdev | 5.86 | 11.53 | 7.77 | 5.13 | 7.51 | 13.00 | 4.00 | 7.55 | 5.29 | 11.06 | 5.00 | 9.54 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1160000.00 | 1220000.00 | 1203333.33 | 1243333.33 | 1525000.00 | 1440000.00 | 1580000.00 | 1630000.00 | 1390000.00 | 1343333.33 | 1470000.00 | 1490000.00 |
| | $\log_{10}(N_o/N)$ | 0.02 | -0.02 | 0.00 | 0.00 | -0.01 | 0.05 | -0.01 | -0.02 | 0.07 | 0.05 | 0.02 | 0.02 |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 30 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | mean plaques | 109.00 | 126.00 | 114.33 | 126.33 | 158.00 | 127.00 | 138.67 | 165.67 | 132.00 | 134.67 | 126.00 | 153.00 |
| | stdev | 1.15 | 4.36 | 9.29 | 6.11 | 1.15 | 11.53 | 8.08 | 3.21 | 77.67 | 18.90 | 7.00 | 7.00 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1090000.00 | 1260000.00 | 1143333.33 | 1263333.33 | 1580000.00 | 1270000.00 | 1386666.67 | 1656666.67 | 1320000.00 | 1346666.67 | 1260000.00 | 1530000.00 |
| | $\log_{10}(N_o/N)$ | 0.05 | -0.03 | 0.02 | 0.00 | -0.03 | 0.10 | 0.05 | -0.03 | 0.09 | 0.05 | 0.08 | 0.01 |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| 40 | STDEV | | | 0.0 | | | | 0.1 | | | | 0.0 | |
| | mean plaques | 119.00 | 121.33 | 120.67 | 122.00 | 156.00 | 130.00 | 165.67 | 152.33 | 128.33 | 123.00 | 122.00 | 147.67 |
| | stdev | 11.53 | 5.69 | 0.58 | | 5.57 | 5.00 | 6.03 | 10.26 | 11.24 | 23.64 | 3.61 | 4.04 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1190000.00 | 1213333.33 | 1206666.67 | 1220000.00 | 1560000.00 | 1300000.00 | 1656666.67 | 1523333.33 | 1283333.33 | 1230000.00 | 1220000.00 | 1476666.67 |
| | $\log_{10}(N_o/N)$ | 0.01 | -0.02 | 0.00 | 0.01 | -0.02 | 0.09 | -0.03 | 0.01 | 0.10 | 0.09 | 0.10 | 0.03 |
| | mean | | | 0.0 | | | | 0.0 | | | | 0.1 | |
| 60 | STDEV | | | 0.0 | | | | 0.1 | | | | 0.0 | |

Table E 5: Effect of EC to MS2 titer

| | Epicatechin 1mM | | | | Epicatechin 2mM | | | | Epicatechin 5mM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| sample | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| Mean plaques | 163.67 | 135.67 | 140.33 | 150.00 | 112.00 | 116.67 | 111.50 | 110.50 | 162.33 | 142.00 | 143.67 | 144.00 |
| stdev | 12.06 | 18.04 | 4.16 | 4.73 | 8.54 | 7.77 | 2.12 | 5.86 | 2.08 | 14.18 | 7.02 | 2.00 |
| dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| pfu/ml | 1636666.67 | 1356666.67 | 1403333.33 | 1500000.00 | 1120000.00 | 1166666.67 | 1115000.00 | 1105000.00 | 1623333.33 | 1420000.00 | 1436666.67 | 1440000.00 |
| $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| STDEV | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| Mean plaques | 149.33 | 140.00 | 147.33 | 137.33 | 97.33 | 142.33 | 118.00 | 110.67 | 128.33 | 118.67 | 117.33 | 142.00 |
| stdev | 14.57 | 17.78 | 19.30 | 12.58 | 23.69 | 5.69 | 16.64 | 8.08 | 3.06 | 1.15 | 20.40 | 7.81 |
| dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| pfu/ml | 1493333.33 | 1400000.00 | 1473333.33 | 1373333.33 | 973333.33 | 1423333.33 | 1180000.00 | 1106666.67 | 1283333.33 | 1186666.67 | 1173333.33 | 1420000.00 |
| $Log_{10}(N_o/N)$ | 0.04 | -0.01 | -0.02 | 0.04 | 0.06 | -0.09 | -0.02 | 0.00 | 0.10 | 0.08 | 0.09 | 0.01 |
| Mean | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| STDEV | 0.0 | | | | 0.1 | | | | 0.0 | | | |
| Mean plaques | 133.33 | 131.67 | 136.33 | 136.00 | 107.00 | 122.33 | 115.00 | 108.00 | 140.00 | 121.33 | 118.67 | 136.33 |
| stdev | 7.37 | 19.14 | 6.03 | 6.08 | 4.36 | 5.86 | 23.52 | 4.00 | 6.24 | 15.50 | 14.84 | 18.15 |
| dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| pfu/ml | 1333333.33 | 1316666.67 | 1363333.33 | 1360000.00 | 1070000.00 | 1223333.33 | 1150000.00 | 1080000.00 | 1400000.00 | 1213333.33 | 1186666.67 | 1363333.33 |
| $Log_{10}(N_o/N)$ | 0.09 | 0.01 | 0.01 | 0.04 | 0.02 | -0.02 | -0.01 | 0.01 | 0.06 | 0.07 | 0.08 | 0.02 |
| Mean | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| STDEV | 0.0 | | | | 0.0 | | | | 0.0 | | | |
| Mean plaques | 156.00 | 158.00 | 162.00 | 145.33 | 93.67 | 119.67 | 122.67 | 107.00 | 138.00 | 103.67 | 125.67 | 137.33 |
| stdev | 30.79 | 18.38 | 42.79 | 5.51 | 10.02 | 8.50 | 6.03 | 11.14 | 15.10 | 12.01 | 12.22 | 10.60 |
| dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| pfu/ml | 1560000.00 | 1580000.00 | 1620000.00 | 1453333.33 | 936666.67 | 1196666.67 | 1226666.67 | 1070000.00 | 1380000.00 | 1036666.67 | 1256666.67 | 1373333.33 |
| $Log_{10}(N_o/N)$ | 0.02 | -0.07 | -0.06 | 0.01 | 0.08 | -0.01 | -0.04 | 0.01 | 0.07 | 0.14 | 0.06 | 0.02 |
| Mean | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| STDEV | 0.0 | | | | 0.1 | | | | 0.0 | | | |
| Mean plaques | 150.50 | 135.67 | 141.00 | 140.67 | 98.50 | 125.67 | 94.67 | 122.67 | 130.50 | 120.33 | 112.00 | 144.67 |
| stdev | 7.00 | 15.53 | 12.17 | 3.51 | 4.73 | 15.95 | 11.68 | 18.58 | 4.73 | 3.51 | 5.57 | 12.50 |
| dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| pfu/ml | 1505000.00 | 1356666.67 | 1410000.00 | 1406666.67 | 985000.00 | 1256666.67 | 946666.67 | 1226666.67 | 1305000.00 | 1203333.33 | 1120000.00 | 1446666.67 |
| $Log_{10}(N_o/N)$ | 0.04 | 0.00 | 0.00 | 0.03 | 0.06 | -0.03 | 0.07 | -0.05 | 0.09 | 0.07 | 0.11 | 0.00 |
| Mean | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| STDEV | 0.0 | | | | 0.1 | | | | 0.0 | | | |
| Mean plaques | 128.50 | 155.67 | 152.67 | 135.67 | 114.00 | 129.33 | 94.00 | 124.33 | 139.67 | 118.33 | 120.67 | 134.33 |
| stdev | 57.28 | 9.87 | 6.66 | 19.22 | 15.00 | 12.22 | 31.00 | 3.06 | 10.60 | 14.05 | 4.62 | 3.51 |
| dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| pfu/ml | 1285000.00 | 1556666.67 | 1526666.67 | 1356666.67 | 1140000.00 | 1293333.33 | 940000.00 | 1243333.33 | 1396666.67 | 1183333.33 | 1206666.67 | 1343333.33 |
| $Log_{10}(N_o/N)$ | 0.11 | -0.06 | -0.04 | 0.04 | -0.01 | -0.04 | 0.07 | -0.05 | 0.07 | 0.08 | 0.08 | 0.03 |
| Mean | 0.0 | | | | 0.0 | | | | 0.1 | | | |
| STDEV | 0.1 | | | | 0.1 | | | | 0.0 | | | |

Table E 6: Effect of EGCg to MS2 titer

| | | EGCg 1mM | | | | EGCg 2mM | | | | EGCg 5.5mM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| contact time(min) | sample | R1 | R2 | R3 | C | R1 | R2 | R3 | C | R1 | R2 | R3 | C |
| | Mean plaques | 137.00 | 139.00 | 139.00 | 133.00 | 181.67 | 197.33 | 186.33 | 194.00 | 273.67 | 247.00 | 257.67 | 273.00 |
| | stdev | 0.00 | 1.41 | 12.73 | 16.97 | 6.43 | 3.51 | 14.64 | 6.56 | 13.80 | 8.89 | 14.57 | 10.00 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1370000.00 | 1390000.00 | 1390000.00 | 1330000.00 | 1816666.67 | 1973333.33 | 1863333.33 | 1940000.00 | 2736666.67 | 2470000.00 | 2576666.67 | 2730000.00 |
| | $Log_{10}(N_o/N)$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Mean | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| 0 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 152.50 | 167.67 | 139.67 | 155.00 | 159.67 | 165.33 | 163.33 | 181.33 | 188.00 | 192.67 | 198.33 | 246.67 |
| | stdev | 88.56 | 13.58 | 5.69 | 89.49 | 6.66 | 11.15 | 2.08 | 7.09 | 17.35 | 11.37 | 10.60 | 13.50 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1525000.00 | 1676666.67 | 1396666.67 | 1550000.00 | 1596666.67 | 1653333.33 | 1633333.33 | 1813333.33 | 1880000.00 | 1926666.67 | 1983333.33 | 2466666.67 |
| | $Log_{10}(N_o/N)$ | -0.05 | -0.08 | 0.00 | -0.07 | 0.06 | 0.08 | 0.06 | 0.03 | 0.16 | 0.11 | 0.11 | 0.04 |
| | Mean | | | 0.0 | | | | 0.1 | | | | 0.1 | |
| 10 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 138.50 | 121.33 | 116.67 | 137.00 | 159.00 | 167.00 | 158.33 | 189.00 | 200.00 | 177.33 | 171.00 | 250.67 |
| | stdev | 17.90 | 13.28 | 6.11 | 79.10 | 7.94 | 5.29 | 3.06 | 5.57 | 115.47 | 6.35 | 8.00 | 32.25 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1385000.00 | 1213333.33 | 1166666.67 | 1370000.00 | 1590000.00 | 1670000.00 | 1583333.33 | 1890000.00 | 2000000.00 | 1773333.33 | 1710000.00 | 2506666.67 |
| | Log10(No/N) | 0.00 | 0.06 | 0.08 | -0.01 | 0.06 | 0.07 | 0.07 | 0.01 | 0.14 | 0.14 | 0.18 | 0.04 |
| | Mean | | | 0.0 | | | | 0.1 | | | | 0.2 | |
| 20 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 93.33 | 112.00 | 111.00 | 142.00 | 139.67 | 141.00 | 140.00 | 169.33 | 184.50 | 150.67 | 186.00 | 260.50 |
| | stdev | 5.03 | 3.61 | 5.57 | 81.98 | 9.45 | 6.00 | 6.56 | 10.97 | 6.35 | 27.43 | 15.39 | 5.86 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 933333.33 | 1120000.00 | 1110000.00 | 1420000.00 | 1396666.67 | 1410000.00 | 1400000.00 | 1693333.33 | 1845000.00 | 1506666.67 | 1860000.00 | 2605000.00 |
| | Log10(No/N) | 0.17 | 0.09 | 0.10 | -0.03 | 0.11 | 0.15 | 0.12 | 0.06 | 0.17 | 0.21 | 0.14 | 0.02 |
| | Mean | | | 0.1 | | | | 0.1 | | | | 0.2 | |
| 30 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 110.67 | 107.67 | 93.67 | 151.00 | 129.67 | 132.00 | 133.67 | 159.67 | 170.00 | 184.33 | 172.00 | 267.67 |
| | stdev | 39.93 | 14.64 | 31.94 | 87.18 | 5.13 | 10.44 | 3.21 | 8.50 | 2.77 | 21.01 | 11.53 | 2.52 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 1106666.67 | 1076666.67 | 936666.67 | 1510000.00 | 1296666.67 | 1320000.00 | 1336666.67 | 1596666.67 | 1700000.00 | 1843333.33 | 1720000.00 | 2676666.67 |
| | Log10(No/N) | 0.09 | 0.11 | 0.17 | -0.06 | 0.15 | 0.17 | 0.14 | 0.08 | 0.21 | 0.13 | 0.18 | 0.01 |
| | Mean | | | 0.1 | | | | 0.2 | | | | 0.2 | |
| 40 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |
| | Mean plaques | 86.33 | 91.33 | 79.00 | 139.00 | 114.67 | 116.33 | 117.33 | 146.00 | 178.00 | 161.00 | 183.67 | 258.67 |
| | stdev | 7.51 | 10.69 | 9.54 | 80.25 | 0.58 | 4.04 | 8.50 | 7.55 | 0.00 | 3.00 | 5.13 | 9.02 |
| | dilution factor | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 | 10^-3 |
| | pfu/ml | 863333.33 | 913333.33 | 790000.00 | 1390000.00 | 1146666.67 | 1163333.33 | 1173333.33 | 1460000.00 | 1780000.00 | 1610000.00 | 1836666.67 | 2586666.67 |
| | Log10(No/N) | 0.20 | 0.18 | 0.25 | -0.02 | 0.20 | 0.23 | 0.20 | 0.12 | 0.19 | 0.19 | 0.15 | 0.02 |
| | Mean | | | 0.2 | | | | 0.2 | | | | 0.2 | |
| 60 | STDEV | | | 0.0 | | | | 0.0 | | | | 0.0 | |

## Appendix F: Supplementary information for hydrogen peroxide determination

## Appendix F1: Raw data from Potassium permanganate titration

| Experiment 1 | | | |
|---|---|---|---|
| permanganate titration | | hydrogen peroxide | |
| Run | Volume titrated | Run | Volume titrated |
| 1 | 10.00 | 1 | 46.00 |
| 2 | 9.50 | 2 | 46.10 |
| 3 | 9.50 | 3 | 46.40 |
| 4 | 9.50 | 4 | 46.50 |
| 5 | 9.50 | Average volume (ml) | 46.25 |
| Average volume (ml) | 9.60 | | |
| | | | |
| wt KMnO4 | 3.211g | | |
| wt Oxalate | 4.502g | | |
| MW oxalate | 90.04 g/mol | | |
| H2O2 wt from 30% stock | 5.0195g | | |
| Experiment 2 | | | |
| permanganate titration | | hydrogen peroxide titration | |
| Run | Volume titrated | Run | Volume titrated |
| 1 | 11.00 | 1 | 46.00 |
| 2 | 11.00 | 2 | 46.00 |
| 3 | 11.20 | 3 | 46.00 |
| 4 | 11.20 | 4 | 46.10 |
| 5 | 11.10 | 5 | 46.00 |
| 6 | 11.00 | 6 | 46.00 |
| 7 | 11.00 | Average volume | 46.02 |
| 8 | 10.80 | | |
| 9 | 11.10 | | |
| Average volume | 11.04 | | |
| | | | |
| wt oxalic acid | 4.5115g | | |
| wt KMnO4 | 3.2004g | | |
| MW oxalate | 90.04 g/mol | | |
| wt H2O2 from 30% stock | 5.0287 g | | |
| Experiment 3 | | | |
| permanganate titration | | hydrogen peroxide titration | |
| Run | Volume titrated (ml) | Run | Volume titrated (ml) |
| 1 | 10.90 | 1 | 46.00 |
| 2 | 10.80 | 2 | 46.20 |
| 3 | 10.90 | 3 | 46.20 |
| 4 | 10.90 | 4 | 46.00 |
| 5 | 10.90 | 5 | 46.20 |
| 6 | 10.70 | 6 | 46.10 |
| 7 | 10.90 | 7 | 46.20 |
| 8 | 10.90 | 8 | 46.10 |
| 9 | 10.80 | Average volume (ml) | 46.13 |
| Average volume (ml) | 10.86 | | |
| | | | |
| wt oxalic acid | 4.5115g | | |
| wt KMnO4 | 3.2094g | | |
| wt H2O2 from 30% stock | 5.0007g | | |

**Appendix F2: Raw data for hydrogen peroxide, individual compound**

Table F 1: Raw data for hydrogen peroxide concentration in Epigallocatechin gallate (EGCg)

| sample | EGCg 62.5 µg/ml | | | | | EGCg 125.0 µg/ml | | | | | EGCg 250.0 µg/ml | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| time (min) | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV |
| 0 | -0.35 | -0.52 | -0.25 | -0.38 | 0.14 | -0.27 | -0.69 | -0.38 | -0.45 | 0.21 | -0.41 | -0.22 | -0.84 | -0.49 | 0.32 |
| 30 | 5.71 | 6.04 | 5.79 | 5.85 | 0.17 | 10.17 | 11.09 | 7.63 | 9.63 | 1.79 | 14.55 | 11.82 | 13.82 | 13.40 | 1.42 |
| 60 | 6.55 | 7.65 | 7.57 | 7.26 | 0.61 | 12.91 | 13.14 | 15.27 | 13.77 | 1.30 | 19.67 | 20.11 | 22.04 | 20.61 | 1.26 |
| 120 | 9.31 | 12.68 | 13.27 | 11.75 | 2.14 | 26.55 | 26.51 | 26.97 | 26.68 | 0.25 | 35.91 | 38.56 | 35.89 | 36.79 | 1.54 |
| 240 | 22.77 | 20.76 | 22.45 | 21.99 | 1.08 | 29.85 | 30.69 | 30.68 | 30.41 | 0.48 | 44.37 | 41.69 | 38.94 | 41.67 | 2.72 |
| 360 | 15.00 | 13.57 | 12.72 | 13.76 | 1.15 | 24.54 | 19.26 | 22.87 | 22.22 | 2.70 | 34.24 | 35.10 | 34.10 | 34.48 | 0.54 |

Table F 2: Raw data for hydrogen peroxide concentration in Gallic acid (GA)

| sample | GA 62.5 µg/ml | | | | | GA 125.0 µg/ml | | | | | GA 250.0 µg/ml | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| time (min) | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV |
| 0.00 | 0.04 | -0.57 | 0.04 | -0.16 | 0.35 | 0.09 | -1.09 | -0.28 | -0.42 | 0.60 | -0.28 | -0.16 | 0.02 | -0.14 | 0.15 |
| 30.00 | 3.96 | 4.78 | 4.82 | 4.52 | 0.49 | 9.48 | 5.59 | 6.22 | 7.09 | 2.09 | 6.09 | 6.34 | 6.34 | 6.25 | 0.14 |
| 60.00 | 7.40 | 7.44 | 7.05 | 7.29 | 0.22 | 8.21 | 9.98 | 9.66 | 9.28 | 0.94 | 8.75 | 9.37 | 10.53 | 9.55 | 0.91 |
| 120.00 | 15.17 | 15.07 | 14.19 | 14.81 | 0.54 | 20.55 | 20.10 | 20.17 | 20.27 | 0.24 | 20.77 | 24.12 | 21.33 | 22.07 | 1.80 |
| 240.00 | 34.98 | 32.62 | 32.81 | 33.47 | 1.31 | 47.44 | 45.75 | 46.41 | 46.53 | 0.85 | 50.87 | 52.70 | 54.13 | 52.57 | 1.63 |
| 360.00 | 25.72 | 27.40 | 25.50 | 26.21 | 1.04 | 42.15 | 41.07 | 39.37 | 40.86 | 1.40 | 55.13 | 49.41 | 52.71 | 52.42 | 2.87 |

Table F 3: Raw data for hydrogen peroxide concentration in Epicatechin (EC)

| sample | EC 62.5 µg/ml | | | | | EC 125.0 µg/ml | | | | | EC 250.0 µg/ml | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| time (min) | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV |
| 0 | 0.02 | -0.17 | 0.11 | -0.02 | 0.14 | -0.77 | 0.45 | 0.35 | 0.01 | 0.68 | -1.38 | 0.14 | 0.34 | -0.30 | 0.94 |
| 30 | 1.07 | 0.90 | 0.81 | 0.92 | 0.13 | 1.09 | -0.05 | 1.69 | 0.91 | 0.88 | 1.61 | 3.52 | 3.67 | 2.93 | 1.15 |
| 60 | 1.21 | 1.64 | 1.71 | 1.52 | 0.27 | 2.68 | 2.69 | 1.89 | 2.42 | 0.46 | 6.79 | 6.21 | 5.13 | 6.04 | 0.85 |
| 120 | 3.17 | 3.17 | 3.14 | 3.16 | 0.02 | 4.85 | 5.09 | 5.03 | 4.99 | 0.13 | 9.26 | 8.44 | 8.15 | 8.62 | 0.58 |
| 240 | 5.24 | 5.22 | 5.22 | 5.23 | 0.01 | 8.22 | 7.87 | 8.91 | 8.33 | 0.53 | 13.44 | 14.76 | 16.38 | 14.86 | 1.47 |
| 360 | 7.45 | 3.91 | 3.99 | 5.11 | 2.02 | 7.56 | 8.02 | 8.33 | 7.97 | 0.39 | 17.52 | 16.44 | 18.16 | 17.37 | 0.87 |

Table F 4: Raw data for hydrogen peroxide concentration in Catechin (C)

| sample | C 62.5 µg/ml | | | | | C 125.0 µg/ml | | | | | C 250.0 µg/ml | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| time (min) | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV | R1 | R2 | R3 | Mean | STDEV |
| 0 | -0.25 | -0.54 | -0.58 | -0.46 | 0.18 | -0.70 | -0.41 | -1.22 | -0.78 | 0.41 | -0.85 | -0.73 | -0.50 | -0.69 | 0.18 |
| 30 | -0.43 | -0.49 | -1.88 | -0.93 | 0.82 | -0.28 | -0.40 | -0.30 | -0.33 | 0.06 | -0.68 | -0.15 | -0.28 | -0.37 | 0.28 |
| 60 | 0.21 | -0.23 | 0.04 | 0.00 | 0.22 | 0.14 | 0.11 | 0.15 | 0.13 | 0.02 | 0.34 | 0.21 | 0.35 | 0.30 | 0.08 |
| 120 | 0.54 | 0.46 | 0.45 | 0.48 | 0.05 | 0.62 | 0.62 | 0.61 | 0.61 | 0.00 | 0.90 | 0.67 | 0.86 | 0.81 | 0.13 |
| 240 | 1.01 | 0.90 | 0.75 | 0.88 | 0.13 | 0.91 | 1.06 | 0.52 | 0.83 | 0.28 | 1.56 | 1.38 | 1.67 | 1.54 | 0.15 |
| 360 | 0.09 | 0.20 | -0.15 | 0.14 | 0.18 | 0.30 | 0.21 | 0.25 | 0.26 | 0.05 | 0.19 | 1.05 | 1.02 | 0.62 | 0.49 |