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The potential of natural products to act as antibiotic resistance breakers against drug-resistant strains of *Escherichia coli* and Methicillin-resistant *Staphylococcus aureus.*

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WARRANTY STATEMENT

Acknowledgements

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Abstract

Drug-resistant bacteria are a major threat to global health. It is known that this is largely due to the widespread use of antibiotics, this has contributed to the emergence of many resistant bacterial strains such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and certain *Escherichia coli* (*E. coli*). This study looked at the potential of natural products to act in combination with penicillin to provide a breaker in the resistance activity against both MRSA and *E. coli*. In total 78 natural products were tested in three different states (fresh, frozen and freeze dried), in three different solvent namely, water, 20% methanol and 20% ethanol. The initial studies used a simple disc diffusion assay to test the natural product alone and in combination with the penicillin against the microbes. Natural products that demonstrated antimicrobial action either alone or in combination, where then examined to determine their minimal inhibitor concentration and final a checkboard assay was carried out. Of the original 78 products, between 50 to 60 of the natural products showed no activity at all depending on the states and extraction method.

So, the most promising result were seen in both bacteria tested against three products namely, tulips, fuchsia and Jasmin. Jasmin and fuchsia were seen to work the best in combination with penicillin against MRSA, whereas tulips demonstrate inhibition against both bacteria on its own.

In conclusion some of the natural product have demonstrated potential as either a new antibiotic or antibiotic breaker, however more evaluation is required to investigate the full potential.

1.0 Introduction

Infections caused by pathogenic microbes such as bacteria and viruses are still one of the most common causes of death in the word today (WHO, 2016). Lower respiratory tract infections are the 4th leading cause of death in the world. Infectious diseases such as pneumonia and influenza remain the deadliest infectious diseases as recorded by the World Health Organisation (WHO) in 2016 (WHO, 2016).

Before the 20th century infections were the leading cause of death and the treatments available were ineffective and life expectancy was just a short average of 40 years (Roser, Ortiz-Ospina and Ritchie, 2020).This was until the unlikely discovery of the world’s first true antibiotic penicillin, by Sir Alexander Fleming in 1928 (Tan and Tatsumura, 2015). However, it was not until 1945 that it become commercially available, its impact was profound and changed the course of medical history, with the beginning of the ‘golden age of antibiotics’. In the years that followed more classes of antibiotics were identified, and this dramatically reduced the mortality rates of bacterial infections worldwide (Adedeji, 2016).

The use of antibiotics is critical to many clinical procedures and many areas in modern medicine rely on them, such as in surgery including organ transplants, bowel resection, hip replacement surgery and caesarean sections, to name but a few. Systemic antibiotic prophylaxis is given before and during these major surgeries to prevent post-operative infections. Without antibiotics the potential of post-surgical infection would be a much higher risk than they are today (WHO, 2018). Antibiotics are fundamental to modern medicine however, extensive misuse of them has resulted in the alarming rise of antimicrobial resistance (AMR) (NICE, 2018).

AMR is one of the biggest threats to global public health, not only to the healthcare industry but also to agriculture and food production. Simply put, AMR is indiscriminate and effects people all over the world regardless of individual circumstance. AMR effects are and will continue to be overwhelmingly profound and there is potential to see a pre-antibiotic era re-emerging (Who.int, 2018). Lord O’Neill estimated in 2016 that by 2050 antimicrobial resistance will cause 10 million deaths and cost £66 trillion (O’Neill, 2016).

AMR became clinically significant just 3 years after the introduction of penicillin, resistance to penicillin was first found in strains of *Staphylococcus aureus* in the early 1940's (Chambers and DeLeo, 2009). Unfortunately, since then with each new antibiotic produced, bacteria have developed resistance mechanisms against them, sometimes as shortly as 2 years after its introduction, as was the case with methicillin (Ventola, 2015). AMR is a natural process which occurs when microbes such as bacteria and viruses evolve to become either partially or fully resistant to antimicrobials this prevents medications such as antibiotics and antivirals working against them, causing the treatment which was once highly effective to become useless. This can lead to persistent life threating infections as well as an increased risk of epidemics and possibly pandemics.

Bacteria have certain mechanisms in which they can become resistant, broadly divided into either be acquired or intrinsic (Hollenbeck and Rice, 2012). Acquired resistance occurs when bacteria gain the ability to respond differently to antibiotics by acquiring resistance to one or several activities in which it used to be sensitive (Hollenbeck and Rice, 2012). There are two mains ways in which bacteria can acquire resistance which is through spontaneous mutations and the acquisition of genes via horizontal gene transfer (Hollenbeck and Rice, 2012). Acquired resistance is mainly seen certain strains or subpopulations of a species for example, *Staphylococcus aureus,* can mutate and become methicillin resistant *Staphylococcus aureus* (MRSA) (Pray, 2008).

Intrinsic resistance is where a bacterial species has the innate ability to resist the activity of certain antimicrobials, which is independent of previous exposure (Amrls.umn.edu, 2019). Natural intrinsic resistance is thought to be due to the following four examples:

- lack of affinity of the drug for the bacterial target
- inaccessibility of the drug into the bacterial cell
- extrusion of the drug by Chromosomally encoded efflux pumps
- innate production of enzymes that inactivate the drug

  (Amrls.umn.edu, 2019).

An example of an intrinsically resistant bacteria is *Pseudomonas aeruginosa*.*(P. aeruginosa)* which has a high intrinsic resistance towards various classes of antibiotics such as aminoglycosides, quinolones and β-lactams (Pang *et al.,* 2019). Intrinsic resistance can be due to biofilm-mediated resistance which can reduce the ability of the antibiotic to reach the bacterial cells, as well as multidrug-tolerant persisted cell formation which are able to tolerate antibiotic action (Pang *et al.,* 2019).

AMR is also attributed by many factors including the overuse or misuse of antibiotics along with the lack of new antibiotic developments. Misuse of antibiotics contributes significantly to the development of resistant bacteria (Ali, Rafiq and Ratcliffe, 2018). Misuse means patients not completing a full course of antibiotics, sharing antibiotics with others, saving antibiotics and taking antibiotics for non-bacterial infections such as for viruses (Ali, Rafiq and Ratcliffe, 2018).

Misuse/overuse of antibiotics is not just prevalent in human medicine but also in veterinary medicine, animal welfare and agriculture. For example, the farming industry will sometimes use antibiotics not just to treat their livestock but also to act as a precaution to prevent infections (Manyi-Loh *et al.,* 2018). This used to be common practice however, more steps have taken place to reduce inappropriate use. The Responsible Use of Medicines in Agriculture (RUMA) are an organisation which have successfully worked to reduce the amount of antibiotics used inappropriately for livestock (RUMA, 2018). Since 2013 the sales of antibiotics in UK for food –producing animals such as beef cattle has fallen by 40%. In 2017 overall use was at 37mg/kg and more recently in 2018 it was revealed that there has been an 53% reduction over 5 years, reaching its lowest point at 29.5mg/kg. It is predicated that this will continue to decline and is seen as an extremely important in the fight against AMR (Ruma.org.uk, 2018).

Another further concern is despite this growing threat of AMR, there has been a significant lack of new antibiotic therapies’ being developed. In contrast to the ‘golden age’ of antibiotics in which 18 pharmaceutical companies were involved in the development of antibiotics up until 1990, in 2013 only four remained invested (ABPI, 2019). Drug development can take many years, resources and is significantly expensive. Due to the nature of antibiotics and the increasing rise of resistance the financial and economic payback is undesirable to large pharmaceutical companies, this is because antibiotics are a short-term course of medication. There are strict guidelines on when they should be prescribed and are sometimes only used as a last resort. Therefore, when compared to lifelong drugs such as insulin they are considered non profitable (Renwick, Brogan and Mossialos, 2016).

In January of 2019, the UK government released a five-year plan to tackle AMR and part of this plan includes expecting pharmaceutical companies to take more responsibility regarding the antibiotic crisis. The government aimed to explore how they can encourage further development of antibiotics by changing the way in which pharmaceutical companies are paid for the drugs they develop, making them more desirable (Gov.UK, 2019). This is extremely important in the fight against AMR, however, more still needs to be done to prevent the world entering a pre-antibiotic era once again.

Two notable species of bacteria which are of highly important when it comes to resistance, are: *S. aureus* and *Escherichia coli.*

1.1 *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus)* a Gram-positive coccus, which is responsible for a wide range of infections, most commonly skin infections but it can also cause food poisoning, bone and joint infections, as well as bacteraemia (NHS, 2018).

S. aureus is very common, it can be found in the environment and is part of the normal flora of skin and in the mucous membranes of healthy individuals. Around 30% of human population are harmlessly colonised with *S. aureus* in their anterior nares, it is usually asymptomatic and does not always lead to infection (Taylor and Unakal, 2019). However, due to its many potential virulence factors such as, hemolysins, proteases and enterotoxins (Easmon and Adlam, 1983). It has high pathogenic potential in its ability to cause disease to a host, particularly if it gains entry to deeper layers of skin tissue and/or the blood circulation (Sakr *et al*., 2018). *S. aureus* is one of the leading causes of nosocomial infections and used to be the most common cause of post-operative wound infections throughout the 2000's, although, currently Enterobacterales have become the most prevalent cause. However, it has been shown that instances of infections caused by *S. aureus* have risen in 2018/2019 when compared to the previous years and remains a significant cause for concern (Assets.publishing.service.gov.uk, 2019; World Health Organisation, 2009).

The normal route of infection by *S. aureus* is acquired from one's own natural flora, for example an open wound site can become colonised with *S. aureus* from a carrier site on the host. The organism can also be acquired from external sources such as other individuals infected bodily fluid or the normal flora from family and healthcare workers. During the early introduction of penicillin in 1943 the mortality rates associated with *S. aureus* infections dramatically reduced. However, *S. aureus* is highly adaptive and was able to quickly overcome the effects of each new antibiotic introduced and rapidly acquired resistance, by 1948 59% of *S. aureus* was resistant to penicillin (Shaffer, 2013).

Penicillin resistance became a major concern in 1950, the need for a new 'magic bullet' was of high priority and in response to this new threat, new antibiotics were discovered and developed, and this led to the introduction of methicillin. Methicillin was produced in 1959 to tackle the resistance to penicillin, however less than one year later some *S. aureus* isolates showed resistance to methicillin which led to the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) (Harkins *et al.,* 2017).

MRSA is resistant to not only methicillin but all β-lactams and their derivatives which include the antibiotic classes' cephalosporins and carbapenems (Fluit and Schmitz, 2003). MRSA carries a gene known as the mecA gene which encodes a penicillin-binding protein 2a (PBP2a). PBPs are essential to the final stages of peptidoglycan biosynthesis for cell wall formation. Beta-lactam antibiotics such as penicillin inhibit the bacterial cell wall biosynthesis, by binding to the PBP's, which is catastrophic to the cell wall structure and will lead to cell lysis and death (Zeng and Lin, 2013). PBP2a has a low affinity for β-lactams and therefore penicillin-based drugs are unable to fully inhibit cell wall biosynthesis and therefore prevents cell death.

1.2 *Escherichia coli*

Escherichia coli (*E. coli*) is a bacterium of the genus Escherichia, it is a Gram-negative bacillus which is part of the normal flora found in the gut of humans and animals (Bélanger *et al.,* 2011). Most strains are not harmful; however, some are highly pathogenic and can cause various intestinal and extra-intestinal infections including urinary tract infections, gastrointestinal infections, cholecystitis, meningitis in new-borns and bacteraemia. *E. coli* is transmitted via the faecal/oral route from contaminated food and water and contaminated items such as kitchen utensils this is due to its ability to survive in the environment for short periods of time (Bush, 2018).

Extra-intestinal pathogenic *E. coli* is the leading cause of nosocomial and community acquired urinary tract infections, as well as the leading cause of bacteraemia and surgical site infections in the UK (Cooper *et al.,* 2019; *Dale et al.,* 2018). Due to the rise in AMR extra-intestinal *E. coli* infections are now a serious global public health concern (Dale and Woodford, 2015).

Some *E. coli* strains produce an enzyme known as extended spectrum beta-lactamases (ESBLs). *E. coli* strains which produce ESBL's are now considered some of the most pathogenic bacteria affecting humans today and pose a significant theat.   ESBL's are enzymes that are responsible for resistance to beta lactam antibiotics such as penicillin and cephalosporins, some strains are however susceptible to beta lactamase inhibitors such as clavulanic acid (Beytur *et al.,* 2015). An enzyme known as CTX-M beta lactamase are the most commonly ESBL produced by *E. coli* and are a significant mechanism of resistance which affects antibiotic resistance today (Gov.UK, 2020). CTX-M's act upon the β-lactam ring, cleaving the amine bond which renders β-lactam antibiotic harmless to bacteria (Bonnet, 2003). Ambler and colleagues (1991) developed a scheme to classify ESBL's in 1991, this divided them into 4 classes from A to D depending on their amino acid sequences. Groups A and C are the most common classes found to be most frequently occurring in bacteria. However, this classification was updated in 2010 by bush and colleagues to group 1 (class c), group 2 (Class A and B) and group 3 (metallo-β-lactamases) (Bush and Jacoby, 2010).

A recent study completed by Alharbi and colleagues (2019) looked at the prevalence of *E. coli* strains resistance to antibiotics in wound infections and raw milk in Saudi Arabia. This was in response to the global widespread occurrence of resistant strains of *E. coli* which included ESBL's in medical, food, and environmental sources. Various samples taken from wound infections resulted in more than 50% of the *E. coli* strains were found to be resistant to; cefazolin, ampicillin, cefuroxime, ciprofloxacin, mezlocillin, moxifloxacin, piperacillin, and tetracycline. In addition, 70% of the isolates from the wound infections were ESBL producing *E. coli* isolates, however 0% of the isolates taken form the raw milk were ESBL's produces (Alharbi et al., 2019).

1.3 Natural Products and their role in defeating AMR

Natural products have played a significant role in the history of medicine and are deeply rooted in many cultures still today across the world. Certain areas of the world the still regularly use natural products such as in East Asia, Africa and South America. In some parts of Africa traditional medicine is the most common form of primary care, the world health organisation describes traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO, Regional office for Africa, 2020).

Due to the rise in antibiotic resistance studies are investigating the potentially properties of traditional medicines. A study by Fuches and colleagues (2018) examined an English medical text from the 10th century known as 'Bald's Leechbook', which contained various plant based medicinal remedies for an array of ailments. Their work examined an eye salve which had antibacterial properties against bacterial infections of the eyelash follicle, also known as a sty. Their work shows that perhaps the use of historical plant based medicinal recipes could be a viable source of treatments in the fight against resistance (Fuches *et al.,* 2018)

Honey is widely used in wound care to promote healing and reduce the risk of infection and has been used for century's as an effective treatment for wound infections (Mandal and Mandal, 2011). Various research has and is continuing to be undertaken with the focus on natural products to either, produce an effective new antibacterial drug or a drug which works in synergy with current antibiotics allowing bacteria to once again become susceptible.

Some natural products are used for their essential oils and others such as turmeric for the chemical components they produce. Turmeric is a common spice originating from India that has been widely recognised for its medicinal properties as well as its culinary uses. Turmeric is a source for polyphenol curcumin, this has been shown to have various health benefits helped by its ability to target multiple signalling molecules such as pro-inflammatory cytokines (Gupta, Patchva and Aggarwal, 2012). Various clinical trials have shown that curcumin has promising results against various co morbidity associated with inflammation, such as Crohn's disease and ulcerative colitis (Gupta, Patchva and Aggarwal, 2012). Curcumin has also shown to have some antibacterial properties against Gram positive and Gram-negative biofilm forming bacteria. Although its effects proved to be more potent when used in conjunction with antibiotics, which indicates that it may be useful as a potential resistance breaker (Kali *et al.,* 2016).

The use of essential oils which are derived from plant-based products are one of the most popular forms of traditional medicine and are used in many ways; not only medicinal uses but for food flavouring and preservation as well as in uses in the cosmetic industries. Commonly used medicinal essential oils are, eucalyptus (respiratory conditions), clove (dental pain), and tea tree oil (Skin conditions) (Ali *et al.,* 2015).

For example, tea tree oil which is commonly used for dermatological complaints has been proven to be an effective treatment for conditions such as acne. In 2018 Mazzarello and colleagues completed a study looking into the effects of a tea tree oil preparation with 20% propolis, 3% "tea tree oil", and 10% "*Aloe vera*" (PTAC) against mild to moderate acne vulgaris when compared with the standard treatment of erythromycin cream. The study showed that the PTAC formula worked better to reduce acne than the erythromycin cream (Mazzarello *et al.,* 2018)

A research study completed by Chovanová and colleagues (2016) investigated the use of the essential oil from *Salvia sclarea* (Clary sage) used in combination with oxacillin against methicillin resistant *Staphylococcus epidermidis* (MRSE). The group demonstrated that on its own Clary sage did not affect the growth of the MRSE, however in combination the oil significantly increased the susceptibility of MRSE to oxacillin. The group determined this was due to the oil modulating the expression of the *mecA* gene in MRSE, causing a down regulation and a reduction in resistance phenotype (Chovanová *et al.,* 2016).

A more recent study conducted on *Melaleuca alternifolia* (tea tree oil) in 2018 by Oliva and colleagues showed potent antibacterial activity when tested against two resistant bacterial strains: methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*. Furthermore, the tea tree oil showed high synergistic effects when combined with antibiotics, this was most prevalent when combined with oxacillin against MRSA (Oliva *et al.,* 2018). Essential oils may very well have a significant impact against resistance however, more research needs to be conducted before this can be certain.

A study completed by Azucena and colleagues (2019) looked at the synergistic effect of curcumin with 12 different antibiotics. They demonstrated increases in the antibiotic's effects against enterotoxigenic *E. coli*, however further studies are required to establish curcumins potential worth in the future as a combination therapy against resistance bacterial strains (Azucena *et al.,* 2019).

Another promising natural product that demonstrates antibacterial properties is verbascoside. Verbascoside is a natural phenylpropanoid glucoside which is found in many varieties of plant species such as *Brassavola cordata (B. cordata),* starflower (*Borage officinalis)*, and common lilac (*Syringa vulgaris)*. Recently a study conducted in 2018 by Bazzaz and colleagues, looked at combining verbascoside with gentamicin against resistant clinical isolates of *S. aureus* and *E. coli*. The study showed that the combination was effective and had synergistic activities against the clinical isolates and were more effective than the antibiotic alone (Bazzaz *et al.,* 2018).

These are just two examples, there are many more natural products which have antibacterial properties on their own and in combination with current antibiotics. More research needs to continue its focus on natural products as the possibilities are seemingly endless and their use in combination with current antibiotics could very well be the key to preventing a pre antibiotic era and break the ever-increasing resistance.

1.4 Antibiotic resistance breakers

Antibiotic resistance breakers (ARB's) are one of the many alternative strategies to fight AMR. A resistance breaker is a method by which a compound is combined with a failing antibiotic to increase its effectiveness and allow it to combat the bacterial resistance mechanisms against them. This is either by enhancing the activity of the antibiotic or acting on the resistance mechanism directly (Laws, Shaaban and Rahman, 2019). ARB's may themselves also have antibacterial properties on their own or only in conjunction with other antibiotics. An example of a current resistance breaker that is already on the market is clavulanic acid. Clavulanic acid is commonly combined with amoxicillin and is a β-lactamase inhibitor. It is semisynthetic and derives from *Streptomyces sp.* It has little antibacterial effect on its own but has strong inhibitory properties against β-lactamases. Clavulanic acid acts in a way to protect the β-lactams from hydrolysis induced by the β-lactamase enzymes (Bush and Bradford, 2016).

Antibiotic research UK are currently focusing on resistance breakers, they are researching various drugs such as antimetabolites, anthracyclines and psychoactive drugs that are on the market today in combination with around 100 different antibiotics against many types of bacteria (Antibiotic research UK, 2020). The potential of ARB'S is an attractive prospect, the possibilities they may bring could aid in slowing the rate of resistance and help to reserve last line antibiotics for urgent and emergent cases only.

1.5 AIM

The aim of this study was to evaluate several natural products and their ability to act as resistance breakers. Various fresh fruit and vegetables will be added to different solvents to create an extraction. The extraction of the natural products will be used to observe whether the products could be used as potential resistance breakers when in combination with penicillin against MRSA and *E. coli*.

2.0 Method & Materials

2.1 Natural product preparation

The natural products were obtained from various places including natural sources, primarily local surrey woodlands and the local supermarket. Table 2.1 shows the source, country of origin and the month they were harvested (if applicable).

Table 2.1. List of Products tested, source, country of origin and month harvested.

	Source	**Country of Origin**	**Month harvested/Brought**
Agapanthus	Effingham	United Kingdom	June
Aquilegia	Weybridge	United Kingdom	June
Alstroemerias	Weybridge	United Kingdom	June
Aran Lilly	Weybridge	United Kingdom	June
Ash Rowan	Bookham	United Kingdom	October
Avocado	Sainsburys	Argentina	November
Baby Corn	Sainsburys	Gambia	November
Birds Eye Chillies	Sainsburys	Thailand	November
Black Pepper Corns	Sainsburys	India	November
Bluebells	Bookham	United Kingdom	April
Blueberries	Sainsburys	Argentina	February
Breakfast Tea	Sainsburys	United Kingdom	February
Bryony	Bookham	United Kingdom	October
Camelia	Effingham	United Kingdom	January
Camomile Tea	Sainsburys	United Kingdom	
Celery	Sainsburys	United Kingdom	February
Chrysanthemums	Effingham	United Kingdom	September
Coffee Beans	Sainsburys	Colombia	February

Cumin	Sainsburys	United Kingdom	November
Dandelion	Neal's Yard Remedies	United Kingdom	November
Dill	Sainsburys	Not Stated	February
Dried apricot	Sainsburys	Turkey	February
Dried cranberries	Sainsburys	United Kingdom	March
Dried oregano	Sainsburys	United Kingdom	March
Dried chives	Sainsburys	United Kingdom	March
Dried curry leaves	Sainsburys	United Kingdom	March
Dried marjoram	Sainsburys	United Kingdom	February
Dried sage	Sainsburys	United Kingdom	February
Dried tarragon	Sainsburys	United Kingdom	February
Duranta	Bookham	United Kingdom	October
Echinacea tea	Neal's Yard Remedies	United Kingdom	November
Elder berries	Bookham	United Kingdom	October
Fresh aubergine	Sainsburys	Belgium	November
Fresh basil	Sainsburys	United Kingdom	November
Fresh coriander	Sainsburys	United Kingdom	November
Fresh curly Parsley	Sainsburys	Canary Islands	February
Fresh rocket	Sainsburys	Italy	February
Fresh spinach	Sainsburys	United Kingdom	March
Fuchsia	Effingham	United Kingdom	June
Goji berries	Neal's Yard Remedies	United Kingdom	November
Grape	Sainsburys	Chile	February
Ground cloves	Sainsburys	United Kingdom	February

Hawthorn	Bookham	United Kingdom	October
Helenium	Effingham	United Kingdom	June
Honeydew Melon	Sainsburys	brazil	October
Jasmine	Effingham	United Kingdom	June
Kale	Sainsburys	Spain	November
Kiwi	Sainsburys	Greece	November
Lavender	Neal's Yard Remedies	United Kingdom	November
Lilacs	Effingham	United Kingdom	June
Mango	Sainsburys	Brazil	February
Marigold	Neal's Yard Remedies	United Kingdom	November
Olive leaf	Neal's Yard Remedies	United Kingdom	November
Papaya	Sainsburys	Brazil	February
Paprika	Sainsburys	United Kingdom	February
Passion fruit	Sainsburys	Colombia	February
Peach	Sainsburys	South Africa	February
Pear	Sainsburys	Belgium	February
Peppermint tea	Sainsburys	England	February
Phlox	Weybridge	England	June
Pineapple	Sainsburys	Costa Rica	November
Pineapple leaves	Sainsburys	Costa Rica	November
Plum	Sainsburys	Chile	March
Poppy Seeds	Sainsburys	United Kingdom	March
Raspberries	Sainsburys	Kenya	March
Red pepper	Sainsburys	Not Stated	November
Raspberry tea	Sainsburys	United Kingdom	October

Runner Beans	Godstone	United Kingdom	June
Slowberries	Bookham	United Kingdom	October
Spindle	Bookham	United Kingdom	October
Spring onions	Sainsburys	Not Stated	October
Strawberry	Sainsburys	United Kingdom	October
Sugar snap Peas	Sainsburys	Colombia	October
Tulips	Weybridge	United Kingdom	March
Thyme	Sainsburys	United Kingdom	April
White Potatoes	Sainsburys	United Kingdom	April
Wild Garlic	Weybridge	United Kingdom	April
Wisteria	Weybridge	United Kingdom	April

The Natural products were first chopped or ground into small pieces and then tested in several different states; fresh/dried, frozen (-80°C) and freeze dried. The entirety of the natural products various parts was used to create the extractions. The frozen products were frozen for up a week before tested. The Freeze dried products where originally chopped or ground when fresh and were freeze dried for a period of 48 hours using a Benchtop Pro freeze drier with Omnitronics The solvents used for the extractions were: methanol (Fisher.UK), ethanol (Fisher.UK) (extracts were prepared in 100% alcohol which was diluted to a final concentration of 20%) and distilled water. For the water extractions 1g of each product was added to 10ml of distilled water at room temperature (ratio of 1:10) and left to infuse overnight in a cool dark place. For both methanol and ethanol extracts 1g of product and 2mls of the relevant solvent was left overnight, following this 8ml of distilled water was then added and agitated to mix. All extractions were carried out in glass bottles. Each extract was then filtered through a jay cloth to remove excess plant material, then through a 0.2µm Micro filter into sterile glass universal bottles. These were then stored in a cool, dry, dark area for no more than seven days.

2.2 Natural product sensitivity test.

Fresh cultures of *E. coli* (NCTC 35218) and MRSA (NCTC 12493) were prepared on nutrient agar (Oxoid, UK) and incubated at 37ºC for 24 hours. Each bacterium was then suspended in 5mls of ringer's solution (Oxoid, UK) to a 0.5 McFarland standard; equivalent to $1.5x10^8$ CFU/ml. The suspension was inoculated aseptically on to 4mm Mueller-Hinton agar plates (Oxoid, UK) using sterile cotton swabs in three different directions. A penicillin disk (1-unit) (Fisher. UK) was applied to one side of each plate, 10µl of the natural product extracts were added on top of the penicillin discs and to the opposite side of the plate (natural product alone). A ciprofloxacin disc (5mg) was used as a positive control and penicillin disc (1-unit) (Fisher, UK) as a negative control. In addition, solvent controls were also conducted for distilled water, ethanol and methanol. For the water control 10 µl drop of distilled water was added to the inoculated agar plate then 10 µl of 20% methanol and 20% ethanol were separately added on to a blank 6mm disc on the same plate. These were then incubated at 37ºC for 24 hours and zones of inhibition were recorded. These tests were repeated in triplicate.

2.3 Minimum inhibitory concentration test

Minimum inhibitory concentration tests (MIC) were performed on products which had given zones of inhibition, both the zones given by the natural product alone and in combination with the penicillin was measured. An aliquot 135 µl double strength Mueller-Hinton broth (Oxoid, UK) was added to the microtiter plate down columns 1-8 and along rows A-H. An aliquot of 135 µl of natural products were added to rows A-H, they were double-diluted down the columns 1-8 in broth with the final 135 µl discarded from the last rows after mixing. Overnight cultures of bacteria was prepared in 5mls of ringers to a 0.5 McFarland standard; equivalent to $1.5x10^8$ CFU/ml, then 100µl of the suspension was further diluted in 900µl ringers, 15 µl of this dilution was then added to all wells to achieve a final concentration of $1.5x10^6$ CFU/ml. Columns 11 and 12 were used for a negative control which contained broth and ringers and a positive control which contained broth and bacteria, well 12B contained broth and the natural product The microtiter plates were then incubated at 37ºC and incubated for 18 hours. The MIC of the natural product was then read visually (by eye) and recorded; all experiments were repeated in triplicate for both bacteria.

2.4 Checkerboard Test of natural products combined with penicillin G

Checkerboard tests were carried out on products which demonstrated a MIC of less than 50% of the natural product concentration, to investigate if the combination of the product and penicillin G had an effect against the bacteria.

0.06.2g of penicillin powder was added to 3mls of double strength Mueller-Hinton broth (Oxoid, UK). An aliquot of 300 µl was filter sterilised and added to 2700 µl of double strength Mueller-Hinton broth (Oxoid, UK). This gave a final concentration of 2048 µg/ml.

An aliquot of 270 µl of the double strength Mueller-Hinton broth (Oxoid, UK) containing penicillin (2048 µg/ml) was added to the microtiter plate at column 1 and down through from rows A-H. An aliquot of 135µl of double strength Mueller-Hinton broth (Oxoid, UK) was then added to across rows 2-8 and columns A-H. The broth and penicillin were then serial diluted across all rows from 1-8. With 135 µl discarded after the final row.

An aliquot of 135 µl of the natural product was added along rows 1-8 and serial diluted down all the columns. An aliquot of 135 µl was the discarded after the final column.

The bacteria were prepared in 5mls of Ringers to a 0.5 McFarland standard; equivalent to $1.5x10^8$ CFU/ml and further diluted to $1.5x10^6$ CFU/ml. An aliquot of 15 µl of bacteria suspension was then added to every well leaving each well with a total volume of 150µl. (Bacterial suspension equivalent to $1.5x10^5$ CFU/ml)

Column 12, rows 1-D were used for controls and included 135 µl of double strength broth alone. 135 µl of the double strength broth and penicillin solution. 135µl of broth and 135 µl of natural product which was then diluted by removing 135 µl and finally 15 µl bacteria and 135 µl of double strength broth. The microtiter plates were then incubated at 37ºC and for 18 hours. The plates were read via a plate reader at 540nm. A printout with each well’s absorbance was obtained. This was repeated in triplicate for both bacteria.

3.0 Results

3.1 Antibiotic sensitivity testing.

All 78 of the natural products were tested against MRSA and *E. coli*, alone and in combination with penicillin G. These products were extracted at a ratio of 1:10 in three solvents, water, 20% methanol and 20% ethanol. Tests were carried out with different percentages of the alcohols against the bacteria to determine which percentage would not cause bacterial cell death. The highest concentration of the alcohol which did not cause bacterial cell death was 20% in the case of both *E. coli* and MRSA. The controls for all tests showed that penicillin on its own against both bacteria produced no ZOI and the average ZOI with ciprofloxacin was 32mm

3.2 Fresh natural products

3.2.1 Activity of fresh natural product against MRSA when extracted in distilled water.

Of the 78 natural products tested against MRSA, 64 produced no ZOI either alone or in combination (see appendix 1a) in their fresh form. A total of two products showed an antibacterial effect alone (without the penicillin), namely, elderberry and peach. The zones of inhibition (ZOI) averages for these products range from 2mm to 4mm, which is relatively small. The standard deviations of these were also quite high indicating a wide range of results across the triplicates. (seen in table 3.1). When combined with penicillin 14 of the natural products produced a ZOI against MRSA in combination with the penicillin, namely; aubergine, basil, black pepper corns, curly parsley, dried chives, dried curry leaves, dried cranberries, dried marjoram, dried oregano, dried sage, dried tarragon, spinach, spring onions and strawberries. The average ZOI for the combination with penicillin ranged from 2mm to 6mm, but again demonstrated high standard deviations (see table 3.1). Three of the products had an effect both on their own and in combination with penicillin, dried curry leaves, dried sage and spring onions. When comparing these alone and in combination there is not much difference in the average ZOI, it is likely that the natural product itself has antibacterial effects and the combination with the penicillin did not have an effect.

<u>3.2.2 Activity of fresh natural product against MRSA when extracted in 20% methanol.</u>

Of the 78 natural products tested against MRSA 68 produced no ZOI either alone or in combination when extracted in 20% methanol (see appendix 1a). Raspberry tea was the only extract which had an effect alone and when combined with penicillin (see table 3.1). Interestingly when alone the raspberry tea produced much larger ZOI (9mm, S.D ±2mm) compared to when it was combination with penicillin G (5mm, S.D ±4mm). It was not clear why this is the case; it is possible the penicillin may have had the opposite effect when in combination thus causing the antibacterial action of the raspberry tea to decline. When combined with penicillin ten products produced a ZOI, namely, baby corn, birds eye chillies, dried thyme, duranta, fuchsia, kiwi, pear, raspberry tea, runner beans and strawberries. The average zones for these products ranged from 2-8mm with standard deviations which range from 0-5mm. A few products namely, strawberry, thyme and kiwi demonstrated large ZOI with a small standard deviation. Kiwi for example had an average ZOI of 7mm with a standard deviation of 0mm. Dried thyme and the strawberry both had average ZOI of 8mm with standard deviation of 1mm.

<u>3.2.3 Activity of fresh natural product against MRSA when extracted in 20% ethanol.</u>

Of the 78 natural products tested against MRSA 65 produced no ZOI either alone or in combination when extracted in 20% ethanol (see appendix 1a). Only two extracts produced a ZOI alone namely; dried marjoram, fuchsia , with an average ZOI ranging from 2mm to 3mm and standard deviations from 3-5mm .In combination with penicillin 13 products produced ZOI namely; birds eye chillies, celery, curly parsley, dried tarragon, dried thyme, grape, hawthorn, paprika, pineapple, plum, raspberry tea and sugar snap peas. The average ZOI for the products in combination ranged from 2mm to 5mm and had high standard deviations. Poppy seeds demonstrated ZOI both alone and in combination with penicillin, both resulting in a ZOI of 3mm and standard deviation of 5mm.

Comparing all 3 extracts although extracts in Water and 20% ethanol produced more results the extracts in 20% methanol had products with large average sizes of ZOI and low standard deviations.

Table 3.1: Average zones of inhibition (ZOI mm) of fresh products against MRSA in water, 20% Methanol and 20% Ethanol extracts. Showing the natural product in combination with penicillin and alone, plus the standard deviation (± SD). N=3

Natural Product	Average zone of inhibition for water extracts (mm)		Average zone of inhibition for Methanol 20% extracts (mm)		Average zone of inhibition for Ethanol 20 % extracts (mm)	
(Fresh)	Product alone	With penicillin G	Product alone	With Penicillin G	Product alone	With Penicillin G
Aubergine		2 (± 3)				
Baby corn				2 (± 4)		
Basil		2 (± 4)				
Birds eye Chillies				3 (± 5)		3 (± 5)
Black pepper corns		2 (± 4)				
Celery						2 (± 4)
Curly parsley		3 (± 5)				2 (± 4)
Dried cranberries		3 (± 5)				
Dried chives		3 (± 5)				
Dried curry leaves	4 (± 5)	5 (± 4)				
Dried marjoram		2 (± 4)			3(± 5)	
Dried oregano		2 (± 4)				
Dried sage	3 (± 5)	3 (± 5)				
Dried tarragon		2 (± 4)				2 (± 4)
Dried thyme				8 (± 1)		5 (± 5)
Duranta				2 (± 4)		
Elderberry	4 (± 7)					
Fuchsia				6 (± 5)	2(± 4)	
Grape						2 (± 4)
Hawthorn						3 (± 5)
Kiwi				7 (± 0)		
Paprika						3 (± 6)
Peach	2 (± 3)					
Pear				3 (± 5)		
Pineapple						3 (± 5)
Plum						5 (± 4)
Poppy seeds					3(± 5)	3 (± 5)
Raspberry tea			9 (± 2)	5 (± 4)		2 (± 4)
Runner bean				3 (± 5)		
Spinach		6 (± 6)				

Spring onions	2 (± 3)	2 (± 4)				
Strawberry		3 (± 5)		8 (± 1)		
Sugar snap Peas						2 (± 4)

<u>3.2.4 Activity of fresh natural product against *E. coli* when extracted in distilled water, 20% methanol and 20% ethanol.</u>

Comparatively when the same fresh products were test against *E. coli* no ZOI were seen in either of methanol and ethanol extract, alone or in combination with penicillin (see appendix 2a). Of the natural produces extract only three of the 78 demonstrate any ZOI, raspberry tea produced a zone of 3mm with a standard deviation of 4mm, alone. When in combination with penicillin just two products produced a ZOI: dried cranberry and birds eye chillies. the average ZOI for the products in combination with the penicillin were 3mm with high standard deviations from 4-6mm (See table 3.2)

Table 3.2: Average zones of inhibition (ZOI mm) of fresh products against *E. coli* in water, 20% Methanol and 20% Ethanol extracts. Showing natural product in combination with penicillin and alone, plus the standard deviation (± SD). N=3

Natural Product	Average zone of inhibition for water extracts (mm)		Average zone of inhibition for Methanol 20% extracts (mm)		Average zone of inhibition for Ethanol 20 % extracts (mm)	
(Fresh)	Product alone	With penicillin G	Product alone	With Penicillin G	Product alone	With Penicillin G
Dried Cranberry		3 (± 6)				
Birds eye Chillies		3 (± 5)				
Raspberry Tea	3 (± 4)					

<u>3.3 Frozen natural products</u>

A total 59 of the products were tested in a frozen form this excludes the dried herbs and spices, 19 in total. The frozen products were tested in the same way as the fresh products each in extracts of water, 20% methanol as well as 20% ethanol. Table 3.3 shows the entire group of frozen products which yielded a ZOI alone and in combination with penicillin against MRSA.

3.3.1 Activity of frozen natural product against MRSA when extracted in distilled water.

Of the 59 natural products tested against MRSA, 51 produced no ZOI either alone or in combination (see appendix 1b). A total of two products when alone produced ZOI in extracts of water, these were: jasmin and tulips. The average zones for these products ranged from 2-3mm with high standard deviations. When in combination with the penicillin six products produced a ZOI: ash rowan, byrony, dried cranberries, duranta, red pepper and tulips. The average ZOI ranged from 2-6mm again with high standard deviations (4-6mm). Tulips produced a ZOI both alone and in combination with an average of 3mm and a standard deviation of 6mm, like the combination result. This perhaps shows that the tulips themselves are having an antibacterial effect on their own.

3.3.2 Activity of frozen natural product against MRSA when extracted in 20% methanol.

Of the 59 natural products tested against MRSA in extracts of 20% Methanol, 48 produced no ZOI either alone or in combination (see appendix 1b). A total of three products produced a ZOI alone namely, fuchsia, jasmin and pineapple. Of these, fuchsia produced an average zone of 8mm with a low standard deviation of 2mm, the other two products (jasmin and pineapple) had an average ZOI of 3mm with standard deviations ranging from 5-6mm. A total pf eight products produced a ZOI in combination with the penicillin namely, alstroemerias, birds eye chillies, dried cranberries, hawthorn, jasmin, peach, plum and spring onion. The average ZOI ranged from 2-3mm and again had high standard deviations jasmin is the only product to have an effect both alone and in combination, however the average ZOI and standard deviations were almost the same with an average of 3mm and a standard deviation of 5mm.

3.3.3 Activity of frozen natural product against MRSA when extracted in 20% ethanol.

Of the 59 natural products tested against MRSA in extracts of 20% Ethanol, 50 produced no ZOI either alone or in combination (see appendix 1b). Only two products produced a ZOI when alone, namely, fuchsia and Jasmin. The average ZOI was 6mm and 2mm respectively and had standard deviations from 4-5mm. When in combination with penicillin seven products produced a ZOI:

alstroemerias, byrony, dried cranberries, jasmin, curly parsley, pineapple leaves and strawberry. The average ZOI ranged from 2- 5mm with an average standard deviation of 4mm. There is not a single product which shows an equal result across the triplicates. However, Jasmin produced a ZOI in every category apart from when in extracts of water and in combination with penicillin.

Table 3.3: Frozen products average zones of inhibition (ZOI mm) against MRSA in combination with penicillin and alone in extracts of water, methanol and ethanol. Showing natural product in combination with penicillin and alone, plus the standard deviation (± SD). N=3

Natural Product	Average zone of inhibition for water extracts (mm)		Average zone of inhibition for Methanol 20% extracts (mm)		Average zone of inhibition for Ethanol 20 % extracts (mm)	
(Frozen)	Product alone	With penicillin G	Product alone	With Penicillin G	Product alone	With Penicillin G
Alstroemerias				3 (±5)		2 (±4)
Ash Rowan		5 (± 4)				
Birds eye chillies				3 (±5)		
Bryony		2 (±4)				2 (±5)
Dried Cranberries		2 (±4)		2 (±4)		5 (±4)
Duranta		6 (±5)				
Fuchsia			8 (±2)		6 (±5)	
Hawthorn				3 (±5)		
Jasmin	2 (±4)		3 (±6)	3 (±5)	2 (±4)	2 (±4)
Curly Parsley						2 (±4)
Peach				3 (±5)		
Pineapple			3 (±5)			
Pineapple Leaves						3 (±5)
Plum				3 (±5)		
Red Pepper		3 (±5)				
Spring Onion				3 (±5)		
Strawberry						5 (±4)
Tulips	3(±6)	4(±6)				

3.3.4 Activity of frozen natural products against *E. coli* when extracted in distilled water, 20% methanol and 20% ethanol.

Of the 59 natural products tested against *E. coli*, 56 produced no ZOI either alone or in combination (see appendix 2b). The results of the frozen products against *E. coli* yielded results only when the extracts were in water, no results were seen when the products were in extracts of 20% Methanol or 20% ethanol. When alone just one product, the tulips produced a result with an average ZOI of 10mm and a standard deviation of 1mm. In combination with penicillin three products showed an antibacterial effect; tulips with and average ZOI result of 12mm and a standard deviation of 1mm, dried apricots with an average ZOI of 3mm and a standard deviation of 5mm and dried cranberries also with an average ZOI of 3mm and a standard deviation of 5mm. Tulips appear to be antibacterial in themselves, however the results do show a marginal increase in the ZOI when in combination with the penicillin of 2mm (See Table 3.4).

Table 3.4: Frozen products average zones of inhibition (ZOI mm) against *E. coli* in combination with penicillin and alone in extracts of water, methanol and ethanol. Showing natural product in combination with penicillin and alone, plus the standard deviation (± SD). N=3

Natural Product	Average zone of inhibition for water extracts (mm)		Average zone of inhibition for Methanol 20% extracts (mm)		Average zone of inhibition for Ethanol 20 % extracts (mm)	
(Frozen)	Product alone	With penicillin G	Product alone	With Penicillin G	Product alone	With Penicillin G
Tulips	11 (± 0)	12 (± 1)				
Dried apricot		3 (±5)				
Dried cranberries		3 (±5)				

3.4 Freeze-dried natural products

Finally, 59 of the products were freeze-dried and tested against MRSA and *E. coli*. The freeze-dried products were tested in the same way as the fresh and frozen products each in extracts of water, 20% methanol as well as 20% ethanol. The results are shown in table 3.5.

3.4.1 Activity of freeze-dried natural products against MRSA when extracted in distilled water.

Of the 59 natural products tested against MRSA in water, 49 produced no ZOI either alone or in combination (see appendix 1c). A total of three products: fuchsia, jasmin and tulips produced a ZOI alone. The average zones were 10mm for the fuchsia, however the standard deviation was quite high at 9mm. Jasmin had an average ZOI of 5mm with a standard deviation of 5mm also. The tulips produced the highest average result of 14mm with a very low standard deviation of 3mm. When in combination with penicillin seven products produced a ZOI: apricot, coriander, fuchsia, jasmine, red pepper, rocket and tulips. Fuchsia and jasmin produced far better average ZOI when in combination with penicillin with fuchsia having an average zone of 10mm and a low standard deviation of 3mm. The jasmin had an average zone of 7mm with a standard deviation of 1mm. This perhaps indicates that the penicillin is helping to increase the antibacterial effect against the MRSA than when they are alone. Tulips produced an average zone of 13mm with a standard deviation of 2mm which is a similar result when it is alone giving the impression that the antibacterial effect is coming from the tulips themselves.

3.4.2 Activity of freeze-dried natural products against MRSA when extracted in 20% methanol.

Of the 59 natural products tested against MRSA in extracts of 20% Methanol, 41 produced no ZOI either alone or in combination (see appendix 1c). A total of three products: fuchsia, Jasmin and raspberries produced a ZOI alone. The fuchsia produced an average ZOI of and standard deviation of 9mm and a standard deviation of 1mm, the jasmin had an average zone of 2mm and a standard deviation of 3mm. finally the raspberries average ZOI was 3mm with a standard deviation of 5mm. When in combination with penicillin a total of 15 products namely: apricot, avocado, baby corn birds eye chillies, byrony, dried cranberries, fuchsia, hawthorn, jasmin, kale, pear, plum, potato, rocket and tulips all produced a ZOI. Except for tulips, fuchsia and Jasmin the average ZOI for these was quite low ranging from 2-5mm with standard deviations ranging from 4-6mm. Tulips produced an average ZOI of 12mm with a standard deviation of just 1mm, Jasmin's average was 7mm with a standard deviation of 2mm and fuchsia 9mm with a standard deviation of 2mm. 41 products produced no ZOI.

3.4.3 Activity of freeze-dried natural products against MRSA when extracted in 20% ethanol.

Of the 59 natural products tested against MRSA in extracts of 20% Ethanol, 50 produced no ZOI either alone or in combination (see appendix 1c). The results showed that three products produced a ZOI alone: Ash Rowan, fuchsia and rocket. The ash rowan had a very small average zone of 2mm with a standard deviation of 4mm however, the fuchsia had a large average ZOI of 11mm with a standard deviation of just 1mm. The rocket produced an average zone of 8mm and standard deviation of 0mm. This indicates that the product may have its own antibacterial effects against MRSA. When in combination with penicillin, six products produced a ZOI: byrony, fuchsia, hawthorn, potatoes, rocket and tulips. The average ZOI ranged from 2-11mm with high standard deviations ranging from 4-7mm. The tulips and fuchsia both produced an average zone of 11mm with low standard deviations of 1-2mm.

For the freeze-dried products again, there is a good result from tulips in all extracts as well as fuchsia which produced a result in every category. Both these products produced large zones of inhibition which were constant along the triplicates. However, these products also showed similar if not the same results when tested on their own. This shows that it is quite possibly the products themselves working against the bacterial than the combination effect with penicillin.

Table 3.5: Freeze dried products average zones of inhibition (ZOI mm) against MRSA in combination with penicillin and alone in extracts of water, methanol and ethanol. Showing natural product in combination with penicillin and alone, plus the standard deviation (± SD). N=3

Natural Product	Average zone of inhibition for water extracts (mm)		Average zone of inhibition for Methanol 20% extracts (mm)		Average zone of inhibition for Ethanol 20 % extracts (mm)	
(Freeze Dried)	Product alone	With penicillin G	Product alone	With Penicillin G	Product alone	With Penicillin G
Apricot		2 (±4)		2 (±4)		
Ash rowan					2 (±4)	
Avocado				2 (±4)		
Baby corn				2 (±4)		
Birds eye chillies				2 (±4)		
Bryony				4 (±6)		4 (±6)
Coriander		2 (±4)				
Cranberries				2 (±4)		
Elderberry						
Fuchsia	10 (±9)	10 (±3)	9 (±1)	9 (±1)	11 (±1)	11 (±2)
Hawthorn				4 (±6)		4 (±7)
Jasmin	5 (±5)	7 (±1)	2 (±3)	7 (±2)		
Kale				2 (±4)		
Pear				2 (±4)		
Plum				5 (±5)		
Potato				3 (±5)		2 (±4)
Raspberries			3 (±5)			
Red pepper		2 (±4)				
Rocket		3 (±5)		3 (±5)	8 (±0)	5 (±4)
Spring onion						
Tulip	14 (±3)	13 (±2)		12 (±1)		11 (±1)

The results of the freeze-dried products against *E. coli* are shown in table 3.6 The freeze-dried products were tested in the same way as the fresh and frozen products each in extracts of water, 20% methanol as well as 20% ethanol.

<u>3.4.4 Activity of freeze-dried natural products against *E. coli* when extracted in distilled water.</u>

Of the 59 natural products tested against *E. coli* in water 52 produced no ZOI either alone or in combination (see appendix 2c). A total of two of the products worked alone against the *E. coli*: birds eye chillies which had an average of 4mm and a high standard deviation of 6mm and tulips which had a very high average ZOI of 11mm and a low standard deviation of 0mm. When in combination with penicillin five products produced a ZOI: celery, coriander, jasmin, spring onion and tulips. The averages range from 2-11mm with mostly high standard deviations ranging from 4-6mm. The tulips had an average ZOI of 11mm and a standard deviation of 0mm, very similar to when the product was on its own.

3.4.5 Activity of freeze-dried natural products against *E. coli* when extracted in 20% methanol.

Of the 59 natural products tested against *E. coli* in extracts of 20% methanol. 55 produced no ZOI either alone or in combination (see appendix 2c). None of the products tested yielded a ZOI against *E. coli* when alone. When in combination with penicillin there were four products which produced a ZOI: blueberry, grape, melon and potatoes. The average ZOI ranged from 2-3mm with standard deviations of 4mm-5mm.

3.4.6 Activity of freeze-dried natural products against *E. coli* when extracted in 20% ethanol.

Of the 59 natural products tested against *E. coli* in extracts of 20% ethanol. 56 produced no ZOI either alone or in combination (see appendix 2c). None of the products tested yielded a ZOI against *E. coli* when alone. When in combination just three products, baby corn, pineapple leaves and potatoes yielded a ZOI however they all had an average ZOI of 3mm with a high standard deviation of 5mm.

Table 3.6: Freeze dried products average zones of inhibition (ZOI mm) against *E. coli* in combination with penicillin and alone in extracts of water, methanol and ethanol. Showing natural product in combination with penicillin and alone, plus the standard deviation (± SD). N=3

Natural Product	Average zone of inhibition for water extracts (mm)	Average zone of inhibition for methanol 20% extracts (mm)	Average zone of inhibition for ethanol 20 % extracts (mm)

(Freeze Dried)	Product alone	With penicillin G	Product alone	With Penicillin G	Product alone	With Penicillin G
Baby corn						3 (±5)
Birds eye chillies	4 (±6)					
Blueberry				3 (±5)		
Celery		2 (±4)				
Fresh coriander		6 (±6)				
Grape				3 (±5)		
Jasmin		3 (±4)				
Honeydew Melon				2 (±4)		
Pineapple leaves						3 (±5)
White potatoes				3 (±5)		3 (±5)
Spring onion		2 (±4)				
Tulip	11 (±1)	11 (±0)				

3.5 Minimum inhibitory concentration (MIC)

Of the original 78 natural products 14 products where taken forward to determine the MIC of the natural product against MRSA, the selection criteria for this was that the natural product produced a zone of inhibition more than once throughout the triplicate. A total of eight fresh, two frozen and four freeze-dried natural products were test.

3.5.1 Determining the MIC of fresh natural product against MRSA when extracted in water, 20% methanol or 20% ethanol

For the natural products extracted in water in their fresh form (see table 3.7) dried oregano gave the lowest MIC of 1.25%, dried marjoram and sage demonstrated a MIC of 2.5% and black pepper corns showed an MIC of 5.0%. Frozen tulips gave an MIC of 1.25% and when the products were freeze-dried coriander and tulips each gave an MIC of 0.625%.

For the natural products extracted in 20% methanol when the natural products were fresh, Thyme gave the lowest MIC of 1.25%, Raspberry tea demonstrated a MIC of 2.5% and Fuchsia showed an MIC of 5.0%. The overall lowest MIC was seen with thyme extracted in 20% ethanol with an MIC of 0.625%. Fuchsia when frozen gave an MIC of 1.25%. For the freeze-dried products apricot gave an MIC of 5.0% and avocado an MIC of 2.5%.

For the natural products extracted in 20% ethanol when the natural products were fresh, just thyme produced an MIC of 0.625%. For frozen products fuchsia had an

MIC of 2.5%. None of the products when freeze-dried produced an MIC of below 5.0%

All the natural product that gave an MIC of greater than 50% are shown in table 8.

Table 3.7: Products with an MIC of less or equal to 50% when tested against MRSA**.**

Natural Product	MIC (%) for water extracts	MIC (%) for 20% Methanol extracts	MIC (%) for 20% Ethanol extracts
Fresh			
Black Pepper Corns	5.0		
Dried Marjoram	2.5		
Dried Sage	2.5		
Fresh Aubergine	5.0		
Dried Oregano	1.25		
Raspberry tea		2.5	
Thyme		1.25	0.625
Fuchsia		5.0	
Frozen			
Tulips	1.25		
Fuchsia		0.113	2.5
Freeze - Dried			
Apricot		5.0	
Avocado		2.5	
Coriander	0.625		
Tulips	0.625		

The products with MIC's greater than 5.0% for MRSA are depicted in table 3.8 in the appendix.

3.5.2 Determining the MIC of fresh natural product against *E. coli* when extracted in water, 20% methanol or 20% ethanol.

For the natural products extracted in water against *E. coli.* The products which showed an MIC of under 5.0% were: Tulips both when Frozen and Freeze-dried demonstrating an MIC of 0.625% for both.

The products with MIC's greater than 5.0% for *E. coli* are depicted in table 3.8.

Table 3.8: Products with an MIC greater than 50% when tested against *E. coli***.**

Fresh Products + water	**Fresh Products + methanol**	**Fresh Products + Ethanol**
Dried Cranberry		
Birds eye Chillies		
Frozen products +Water	**Frozen products + methanol**	**Frozen products + Ethanol**
Dried Apricot		
Dried Cranberries		
Freeze-dried Products + Water	**freeze-dried Products + Methanol**	**freeze-dried Products + Ethanol**
Celery	Blueberry	Baby Corn
Spring Onion	Grape	Pineapple Leaves
Coriander	Melon	Potato
Jasmin	Potato	

3.6 Checkerboard test of natural products combined with penicillin G.

Initially a single well checkboard test was performed with the highest concentration of natural product at 5.0% of its MIC and penicillin at 1024mg/l. This was due to time constraints, it was decided that the highest combination of both the natural product and the antibiotic to see if inhibition inn growth could be seen, If growth was seen at these concentrations, they were not taken forward for full checkboard testing. If inhibition however was observed these products were taken forward for a full checkerboard test. This resulted in 11 checkerboard tests for the MRSA and one for *E. coli.*

The checkerboard results were determined by eye looking for bacterial growth. The checkerboards were also plated to confirm growth and back up the visual results. The individual wells were plated on Muller Hinton agar. The minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC) were also determined for those that gave results.

The results against MRSA showed that the dried curry leaves and curly parsley in extracts of water had bacterial growth in all wells, this was seen visually and when plated. The plated bacteria showed that the growth amount was the same in all wells. For the extracts in methanol, duranta in its fresh form also visually showed growth and when plated. Fuchsia had varied results with the first run visually showing no growth along the entire first row showing a checkerboard MIC of 8 µg/ml of the penicillin with the extract at 5.0%, the MBC was the same in this case. However subsequent runs 2 and 3 showed all growth and the bacterial growth was consistent across all wells.

For the extracts in ethanol, fresh plum, dried cranberries and frozen strawberry and freeze-dried rocket all showed growth in the wells both visually and when plated. The fuchsia in freeze-dried form in run 1 gave a checkerboard MIC of 256 µg/ml of the penicillin with the extract at 5.0%, for run 2 the MIC was 128 µg/ml of the penicillin with the extract at 5.0% again, this was also seen visually. The MBC for both was the same as the MIC. The third run however showed full growth both visually and when plated. Finally, the tulips in freeze-dried form gave an MIC of 1024 µg/ml of the penicillin with the extract at 5.0% in run 1, in run 2 interestingly the MIC was 8 µg/ml of the penicillin with the extract at 2.5%. Finally, the third run gave an MIC of 8 µg/ml of the penicillin with the extract at 5.0%. The MBC was the same for all 3 runs.

The coriander in water against *E. coli* showed all growth both visually and when plated across all 3 runs.

4.0 Discussion

The aims of this study were to investigate whether the use of natural products could potentially act as an antibiotic breaker when use combination with penicillin against beta lactam resistant *E. coli* and MRSA. The natural products were tested in three different forms, namely, fresh, frozen (-80) and freeze-dried. These products were also extracted in three different solvents, namely, distilled water, 20% methanol and 20% ethanol.

A large majority of the products tested provided no evidence to suggest they were effective either alone or as a resistance breaker, against MRSA and *E. coli.* This was the case for the fresh, frozen and freeze-dried products in each of the extracts. The controls for all tests showed that penicillin on its own against both bacteria produced no ZOI.

4.1 Antimicrobial properties of fresh products alone and in combination with penicillin against MRSA and *E coli.*

When looking at the fresh products tested against MRSA of the 78 products tested; 62, 68, 63 (water, methanol and ethanol respectively) provided no result either alone or in combination with penicillin G. When the same products were tested against *E. coil*; 76 products did not have any effect when in extracts of water and all 78 products showed no activity when in extracts of methanol or ethanol.

4.2 Fresh products extracted in distilled water

Of the 16 products which gave a result against MRSA when extracted in water; two products produced a ZOI alone (elderberry and peach) and 14 produced a ZOI when in combination with penicillin (aubergine, basil, black pepper corns, curly parsley, dried cranberries, dried chives, dried curry leaves, dried marjoram, dried oregano, dried sage, dried tarragon, spinach, spring onions and strawberries).

At the time of writing, this is the first time these products noted above have been tested using a combination of antibiotic and the whole natural product extracted in water against MRSA. However, there are similar studies which have shown antibacterial properties of some of the above products despite using different methods, including various extraction methods and use of the natural product, being that most use the essential oil of the products rather than the product as a whole.

A previous study has shown that various parts of the aubergine, including the roots, stems, leaves, flowers and fruits, the sperate parts of the aubergine were dried individually and 20g of the dried product was mixed with distilled water, blended down and filter sterilised then the extract was concentrated by drying at room temperature. Once dry various amounts of product were added to distilled water which was added to the bacteria using the Well agar diffusion method. The results showed that the antibacterial effects of all extracts varied depending on strain of bacterial and plant parts. The roots provided the largest ZOI against *S. aureus* and *E. coli* (Al-Janabi and Al-Rubeey, 2010). Whilst it has been previously shown to work its own, this current study showed that it did not work alone and only in combination with penicillin, however the roots were not used in this study, as just the flesh of the aubergine was used as we were limited to what we could get from the local supermarket.

Basil, oregano, and tarragon are other products previously shown to have antimicrobial effects, however, this is with regards to their essential oils rather than the whole plant themselves (Sakkas and Papadopoulou, 2017), (Lu *et al*., 2018), (Chaleshtori *et al.,* 2013). In this current study basil, oregano and tarragon only produced results when in combination with penicillin.

Black pepper corns also have been shown to possess some antibacterial properties when extracted in chloroform against *E. coli* and *S. aureus* (Zou, Hu and Chen, 2015). This was not seen in the current study and no ZOI were seen when the product was alone. However, the extraction methods were different.

Elderberry has been shown in by Krawitz and collagues (2011), to have antibacterial effects against *Streptococcus pyogenes* and *Branhamella catarrhalis*, using liquid extract of the elderberry rather than the whole berry. (Krawitz *et al.,* 2011). This current study shows antibacterial activity alone against MRSA but not in combination.

Interestingly there were no current studies found that indicated any species of peach as having shown any prior antibacterial effects, in this current study it was shown to have activity against MRSA on its own.

A recent study by Aslam and colleagues in 2017, used a similar method to determine the antioxidant activity, anti-inflammatory activities, anti-cancer and chemical composition of spring onion (*Allium fistolisum*) extracts. They also used water, methanol and ethanol extracts. A total of 100mls of the solvent was used with 10g of the product, however the preparation of the natural product was different, they used the leaves and the bulb of the plant separately, whereas in the current work they were combined. They found that in each of the extracts produced for *S. aureus, E. coli, Pseudomonas aeruginosa* and *Bacillus cereus* (Aslam *et al.,* 2017). Although similar in method the results gained by Aslam and colleagues differ from those in this current study, in which there was no activity shown when the spring onion was in extracts of methanol and ethanol and only when in extracts of water. The addition of penicillin showed no increase in the antibacterial action, therefore the ZOI were solely due to the antimicrobial products of the natural product itself.

A study like this one by Hanan and colleagues (2016) showed that curry leaves have a very potent antimicrobial effect, then extracted methanol and ethanol against *Staphylococcus sp., E. coli, Streptococcus* sp. *and Proteus* sp. However, this current study did not collaborate these findings, the result only showed antibacterial properties against MRSA when in extracts in distilled water, both in combination with penicillin and alone. The curry leaves showed no effect on the bacteria when in extracts of methanol and ethanol (Hanan, Ifran and Ali, 2016).

4.3 Fresh products extracted in 20% methanol

When the fresh products were extracted in methanol one product produced a ZOI when alone (Raspberry tea) and 10 of the products produced a ZOI when in combination with penicillin (baby corn, birds eye chillies, dried thyme, duranta, fuchsia, kiwi, pear, raspberry tea, runner beans and strawberries). To the authors knowledge there are no studies similar which indicated the antibacterial nature of the above products in extracts of methanol.

4.4 Fresh products extracted in 20% ethanol

The fresh extracts in ethanol showed that of the 15 products which produced a ZOI; two produced a ZOI alone (dried marjoram and fuchsia) and 13 in combination with penicillin (birds eye chillies, celery, curly parsley, dried tarragon, dried thyme, grape, hawthorn, paprika, pineapple, plum, raspberry tea and sugar snap peas). To the authors knowledge there are no studies similar which indicated the antibacterial nature of the above products in extracts of ethanol.

Of the three fresh products which yielded a result against *E. coli* when extracted in water one product produced a ZOI of inhibition alone (Raspberry tea) and the two when in combination with penicillin (dried cranberries and birds eye chillies) The above products have not been previously shown in prior studies to have antibacterial effects in combination with antibiotics or alone with the exception of birds eye chilli. Chills contain high levels of capsaicin and when testing against various bacteria, serval studies have shown that capsaicin does have antibacterial activity. For example, a study conducted in 2016 by Sen and colleagues in which they tested various chillies, including birds' eyes showed activity against both gram-negative and gram-positive bacteria. They used a similar extraction method to this study where they took the fruit of the plant ground it into a paste and added 5g to 20mls of water and methanol however they followed on with putting the extracts in a rotary shaker for 48 hours then they took the extracts and filtered and concentrated them in vacuum at minimum pressure using rotary evaporator (Sen *et al*., 2016).

4.5 Antimicrobial properties of frozen products alone and in combination with penicillin against MRSA and *E coli*

This is the first time to the best of the authors knowledge, that the following frozen products, extraction techniques and testing methods used have demonstrated antibiotic activity either alone or in combination against MRSA and *E. coli* results.

When looking at the frozen products against MRSA, only 59 of the 78 products could be tested, as explained previously not all the products were suitable to be frozen as they were already dried. The 19 products that where did not undergo this process were brought dried and therefore it was felt that insufficient ice crystals would be formed to rupture the membrane of the plants during the freezing process. No result was seen either alone or in combination with penicillin G for 52, 48, 51 products (water, methanol and ethanol respectively). When the same 59 products were tested against *E. coil* three of the waters extracts showed activity either alone or in combination however the remaining amount of products; 56, 59, 59 (water, methanol and ethanol respectively) provided no result either alone or in combination with penicillin G.

Of the three frozen products which yielded a result against *E. coli* when in extracts of water one product produced a zone when alone (tulip) and all three did when in combination with penicillin (tulips, dried cranberries and dried apricot).

When extracted in water seven of the products yielded a result against MRSA with two producing a ZOI alone (Jasmin and tulips) and six when in combination with penicillin G (ash rowan, byrony, dried cranberries, duranta, red pepper and tulips.

When the products were extracted in 20% methanol 10 of the products produced a ZOI against MRSA, of these, three worked alone (fuchsia, jasmin and pineapple) and eight worked in combination with penicillin (alstroemerias, birds eye chillies, dried cranberries, hawthorn, jasmin, peach, plum and spring onion).

Finally, eight products produced a ZOI against MRSA when in extracts of 20% ethanol, with two of these producing a ZOI alone (fuchsia and jasmin) and seven when in combination with penicillin (alstroemerias, byrony, dried cranberries, jasmin, curly parsley, pineapple leaves and strawberry).

4.6 Antimicrobial properties of freeze-dried products alone and in combination with penicillin against MRSA and *E coli.*

This is the first time to the best of the authors knowledge, that the following freeze-dried products, extraction techniques and testing methods used have demonstrated antibiotic activity either alone or in combination against MRSA and *E. coli* results.

When looking at the freeze-dried products against MRSA 59 of the 78 products were able to be freeze-dried, the remaining 19 samples where already dried. No result was seen either alone or in combination with penicillin G for 52, 43, 52 (water, methanol and ethanol respectively) against MRSA. When the same products were tested against *E. coil*; 53, 55, 56 (water, methanol and ethanol respectively) provided no result either alone or in combination with penicillin G.

When extracted in water seven products yielded a ZOI against MRSA with three of those alone (fuchsia, jasmin and tulips) and all seven when in combination with penicillin (apricot, coriander, fuchsia, jasmine, red pepper, rocket and tulips). Next, 16 of the products extracted in 20% methanol produced a ZOI, Three of these when alone (fuchsia, Jasmin and raspberries) and 16 when in combination with penicillin(apricot, avocado, baby corn, birds eye chillies, byrony, dried cranberries, fuchsia, hawthorn, jasmin, kale, pear, plum, potato, rocket, spring onion and tulips. Finally, seven of the products produced ZOI when in extracts of 20% ethanol, Three when alone (ash Rowan, fuchsia and rocket) and six when in combination with penicillin (byrony, fuchsia, hawthorn, potatoes, rocket and tulips).

When the same products where tested against *E coli* six products in extracts of water produced a ZOI, two alone (birds eye chilies and tulips) and five in combination with penicillin (celery, coriander, jasmin, spring onion and tulips). When in extracts of 20% methanol four products produced a ZOI with none working alone and all four working in combination with penicillin (blueberry, grape, melon and potatoes). Finally, when the products were in extracts of 20% ethanol yielded only three which produced a ZOI and all of these where when in combination with penicillin (baby corn, pineapple leaves and potatoes).

Throughout the results five products produced a result in all forms against MRSA (Fresh, frozen and freeze – dried) these were birds eye chilies, dried cranberries, hawthorn, plum and spring onion. When the products were fresh there were very few similarities between all the forms results, in fact only one result was consistent with the frozen and freeze-dried products (birds eye chilies in methanol when combined with penicillin). However comparatively the frozen and freeze-dried forms showed to have very similar results with all five of the products producing a ZOI when extracted in methanol and in combination with penicillin. Looking at the ZOI and comparing them, the frozen products average ZOI for all the products was around 3mm with an average standard deviation of 5mm. The freeze-dried products had an average of 2mm and an average standard deviation of 5mm. However, there is only a difference of 1mm between the ZOI's and they have the same average standard deviations so this cannot be definitively proven when looking at these results. The results from these five products do however indicate that alcohol solvents could potentially be increasing the antibacterial action, particularly the methanol.

There could be many variables which effect the results when using natural products and different solvents.

Although a lot of the results have shown very small ZOI (mm) this is considered a result because these results are an average of triplicates. Quite often there would be a ZOI recorded in the first run and not in the second or third run (or variations of these). This caused the average result to reduce to smaller diameters than the penicillin discs themselves.

4.7 Alcohol polarity

Water, methanol and ethanol are all polar solvents, they contain bonds between atoms which have a difference in electronegativity, such as oxygen and hydrogen. Non- polar solvents are the opposite and have bonds between atoms with very similar electronegativities.

Most studies similar to this one tend to use polar solvents. The use of a solvent will influence any chemical reactions that occur as well as influence the properties of substances, this can include solubility and reaction rates. Therefore, the choice of solvent combined with the natural products can have a significant effect on its antibacterial ability, either enhancing or reducing them. Polar solvents are much more likely to participate and enhance a reaction and therefore the use of water, methanol and ethanol were important in order to compare the results and observe whether or not the use of a solvent other than water would help to increase the natural products antibacterial action (Ashenhurst, 2020).

It appears that based on the results gained throughout this study that there is some increase in activity when the alcohol solvents were used, this was true of tulips, Jasmin and fuchsia. Tulips, jasmin and fuchsia each showed no activity when in extracts of water, but all produced a ZOI against the bacteria when in 20%methanol or 20%ethanol. The type of solvent that should be combined with each natural product requires more research to determine how best to either enhance or kick start the antibacterial activity.

4.8 Regionality, seasonality and growing methods and their effects upon antibacterial efficacy

Something to consider when looking at the results is the source of the natural products themselves, where the come from, when they were grown, in what season they were harvested and how were the processed. Many of the products were obtained from local sources such as common woodland (Surrey) and gardens, these products were only available depending upon the season. All plants and berries were harvested in autumn and spring, this includes the Ash rowan, Duranta, elderberry, hawthorn, spindle, agapanthus aquilegia, alstroemerias, aran Lilly, bluebells, bryony, fuchsia, Chrysanthemums, Helenium, slow berries and lilacs. However other product where obtained from supermarkets and originated from a vast array of countries and seasons. In addition, these products were also transported and stored before becoming available in the supermarket. This is of course due to the limitations that is not easy to overcome in the UK climate, as certain fruits and vegetables used in this study cannot be easily grown here. These factors could all have had influence the antibacterial activity of the natural products or their concentration in the plants (Castelo, Menezzi and Resck, 2012)

A study conducted in 2012 by Castelo and colleagues showed that seasonality influenced the production and composition of essential oils. They showed that different seasons influenced activity levels in the production of essential oils. The levels tended to increase during the dry seasons of summer and spring and reduced during winter and autumn (Castelo, Menezzi and Resck, 2012). Although antibacterial tests were not carried out Castelo and colleagues did show that seasonality is a factor that must be considered.

A more recent study by López-Romero and colleagues in 2018, looked at the seasonal effect on the biological activities of Mexican bay leaf (*Litsea glaucescens*) extracts against *S. aureus* and *E. coli*. This study by López-Romero and colleagues also showed that the different seasons did influence the concentration of phenolic compounds found within the extracts as well as other biological activities the product possesses. Their study also investigated the antibacterial effects and the results showed that there was a moderate increase in activity when the product was obtained in autumn and summer (López-Romero *et al.,* 2018). Seasonality is a factor that should be considered, however due to the constraints of the seasons in context with time frame of the research in this current study, it was not possible to fully consider seasonality of all the products tested. However, more testing on these products would need to be completed before seasonality is considered a factor in the results.

4.9 Natural products prepared in different states: fresh frozen and freeze-dried

Each of the products were extracted in their original form or 'fresh' form, those that could be were also frozen and freeze- dried. This was to see whether changing the composition of the natural product would enhance the result of an active compound being released from the plant material. Most products showed no change at all however some did show some slight changes when compared between fresh, frozen and freeze- dried forms. The most notable being tulips, Jasmin and fuchsia all of which showed no antibacterial effect when in their fresh form in any of the extracts against both bacteria's. However, all three started to show activity when frozen which increased even further when they were freeze-dried. This gives some indication that the active components in these products which are antibacterial seem to increase or are being released when they are altered by freezing or freeze- drying the products. This could be due to the formation of ice crystals which form during the freezing process which in turn ruptures the membrane of the plant which may result in increased release of the antibacterial components. Similarly, with freeze-dried products in which the water content is removed this may reduce the chance of the dilution of potential antibacterial compounds which in some cases may allow increased antibacterial concentrations.

4.10 Further work and improvements.

There are several areas were further investigating should be carried out, particularly when looking at the results from tulips, fuchsia and jasmin. Each have shown some potential when in combination with penicillin against both MRSA and *E. coli.* However, a lot more needs to be done to investigate what antibacterial compounds they hold, their mechanism of action and whether these can be enhanced by using a combination therapy with antibiotics.

One potential further methods would be to look at the method of extraction, it may be worth including hot water extracts, as a study completed in 2013 by Khan and colleagues showed some success against *B. subtilis, S. aureus, E. coli, P. vulgaris, P. aeruginosa*. They tested the antibacterial activity of three medicinal plants: *Bergenia ciliata*, *Jasminum officinale*, and *Santalum* album, using both cold water and hot water extracts. For the hot water extracts they added the dried powdered of each plant to distilled water and heated it in an incubator to 60 °C for a period of 24 hours. The results showed that *Bergenia ciliate* produced ZOI against each bacterial listed, however the cold-water extracts produced slight larger zones. For *Jasminum officinale* it only produced a ZOI against *P. aeruginosa* when in hot water extracts, however again the cold-water extracts produced much larger zones than the hot water extracts. Finally, Santalum album produced zones against *P. aeruginosa* and *P. vulgaris* when in hot water extracts against the zones were not as large as those extracts in cold water. Although the study shows that there is a decrease in the ZOI in the hot water extracts, the products were not tested in combination with penicillin, so it cannot be ruled out that the combination could enhance the antibacterial properties (Khan *et al.,* 2013)

The use of different solvents may also be indicated, a study which included a similar method to this one has used chloroform as well as ethanol (Pangilinan *et al.,* 2018). They tested the antibacterial activity of *Spathiphyllum cannifolium* and the results showed that the chloroform extracts inhibited the growth of *E. coli, S aureus, B. subtilis,* and *P. aeruginosa* whereas the ethanol extracts showed antibacterial activity against just the *E. coli, S. aureus and B. subtilis.* The result also showed that although the ZOI where slight smaller against *S. aureus* and *B. subtills* they had a lower standard deviation. They concluded that chloroform is the better solvent for the extraction of antibacterial compounds when compared with ethanol. (Pangilinan *et al.,* 2018). A further extraction method that could be explored would be extracting the natural product using a SOXTHERM which will allow the use of hot alcohol extracts under reflux.

As well as other methods of extraction, it is important to investigate the potential compounds that may be responsible for the antibacterial activity of the natural product and trying to isolate them. One way this could be done is by using thin layer chromatography (TLC). TLC can be used to separate constituents from natural products in isolation and determining molecular weight range. TLC is used often to separate secondary metabolites such as amino acids, proteins, polyphenols and flavonoids and when combined with agar-overlay containing bacteria it can show the size of the component which is aiding the antibacterial action of the natural product. Which can then be taken forward to identify the components using further methods such as DOSY NMR (diffusion ordered spectroscopy - nuclear magnetic resonance), which can identify the chemical components and structures of the individual compounds within the raw natural product (Gresley *et al.,* 2012).

4.11 Conclusion

There is a lot of potential in natural products to aid in the effort to reduce microbial resistance. For example, tulips provided large ZOI against bot MRSA and *E. coli*, this was mainly seen when the product was on its own, this product has not been shown tested before in this manner. With regards to the combination several the products in this study were shown to be antibiotic breakers, however due to larger standard deviations further work needs to be carried out to investigate whether these products have any potential as resistance breakers. This study has shown that it may be possible that a combination of natural products and antibiotics may provide a break in resistance.

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6.0 Appendices

APPENDIX 1a - Fresh natural products which produced no ZOI against MRSA either alone or in combination with penicillin G when extract in water, methanol 20% or ethanol 20%

Fresh natural extracted in a solvent of		
Distilled water	Methanol 20%	Ethanol 20%
Agapanthus	Alstroemerias	Agapanthus
Aquilegia	Agapanthus	Aquilegia
Alstroemerias	Aran Lilly	Alstroemerias
Aran Lilly	Ash Rowan	Aran Lilly
Ash Rowan	Aubergine	Ash Rowan
Avocado	Avocado	Avocado
Baby Corn	Basil	Baby Corn
Bluebells	Black pepper corn	Black Pepper Corns
Blueberries	Blueberry	Bluebells
Breakfast Tea	Byrony	Blueberries
Bryony	Camelia	Breakfast Tea
Camelia	Camomile	Bryony
Camomile Tea	Celery	Camelia
Celery	Birds eye chillies	Camomile Tea
Chrysanthemums	Chrysanthemums	Chrysanthemums
Coffee Beans	Coffee beans	Coffee Beans
Cumin	Coriander	Cumin
Dandelion	Cranberry	Dandelion
Dill	Cumin	Dill
Dried Apricot	Curry leaves	Dried Apricot

Dried Chives	Dandelion root	Dried Cranberries
Duranta	Dried Apricot	Dried Oregano
Echinacea tea	Dried Chives	Dried Chives
Fresh Coriander	Dried Sage	Dried curry leaves
Fresh Rocket	Echinacea Tea	Dried Sage
Fuchsia	Goji berries	Duranta
Goji Berries	Grape	Echinacea tea
Grape	Hawthorn	Elder berries
Ground Cloves	Helenium	Fresh Aubergine
Hawthorn	Jasmin	Fresh Basil
Helenium	Kale	Fresh Coriander
Honeydew Melon	Lavender	Fresh Rocket
Jasmine	Aran Lilly	Fresh Spinach
Kale	Mango	Goji Berries
Kiwi	Marjoram	Ground Cloves
Lavender	Marigold	Helenium
Lilacs	Melon	Honeydew Melon
Mango	Olive leaf	Jasmine
Marigold	Oregano	Kale
Olive Leaf	Pineapple	Kiwi
Papaya	Pineapple leaves	Lavender
Paprika	papaya	Lilacs
Passion Fruit	Paprika	Mango
Pear	Parsley	Marigold
Peppermint Tea	Peach	Olive Leaf
Phlox	Peppermint Tea	Papaya
Pineapple	Phlox	Passion Fruit

Pineapple Leaves	pineapple	Peach
Plum	Plum	Pear
Poppy Seeds	Poppy Seeds	Peppermint Tea
Raspberries	Raspberry	Phlox
Red Pepper	Red Pepper	Pineapple Leaves
Raspberry Tea	Rocket	Raspberries
Runner Beans	Runner Bean	Red Pepper
Slowberries	Spring onion	Runner Beans
Spindle	Spinach	Slowberries
Sugar snap Peas	Spindle	Spindle
Tulips	Sugar snap peas	Spring Onions
Thyme	tulip	Strawberry
White Potatoes	White Potatoes	Tulips
Wild Garlic	Wild Garlic	Thyme
Wisteria	Wisteria	White Potatoes
		Wild Garlic
		Wisteria

APPENDIX 1b -Frozen natural products which produced no ZOI against MRSA either alone or in combination with penicillin G when extract in water, methanol 20% or ethanol 20%

Frozen natural extracted in a solvent of		
Distilled water	Methanol 20%	Ethanol 20%
Agapanthus	Agapanthus	Agapanthus
Aquilegia	Aquilegia	Aquilegia
Alstroemerias	Aran Lilly	Aran Lilly
Aran Lilly	Ash Rowan	Ash Rowan
Avocado	Avocado	Avocado
Baby Corn	Baby Corn	Baby Corn
Birds Eye Chillies	Bluebells	Birds Eye Chillies
Bluebells	Blueberries	Bluebells
Blueberries	Bryony	Blueberries
Camelia	Camelia	Camelia
Celery	Celery	Celery
Chrysanthemums	Chrysanthemums	Chrysanthemums
Dill	Dill	Dill
Dried Apricot	Dried Apricot	Dried Apricot
Elder berries	Duranta	Duranta
Fresh Aubergine	Echinacea tea	Elder berries
Fresh Basil	Elder berries	Fresh Aubergine
Fresh Coriander	Fresh Aubergine	Fresh Basil
Fresh curly Parsley	Fresh Basil	Fresh Coriander
Fresh Rocket	Fresh Coriander	Fresh Rocket
Fresh Spinach	Fresh curly Parsley	Fresh Spinach
Fuchsia	Fresh Rocket	Grape

Grape	Fresh Spinach	Hawthorn
Hawthorn	Grape	Helenium
Helenium	Helenium	Honeydew Melon
Honeydew Melon	Honeydew Melon	Kale
Kale	Kale	Kiwi
Kiwi	Kiwi	Lilacs
Lilacs	Lilacs	Mango
Mango	Mango	Papaya
Papaya	Papaya	Paprika
Passion Fruit	Paprika	Passion Fruit
Peach	Passion Fruit	Peach
Pear	Pear	Pear
Phlox	Phlox	Phlox
Pineapple	Pineapple Leaves	Pineapple
Pineapple Leaves	Raspberries	Plum
Plum	Red Pepper	Poppy Seeds
Raspberries	Runner Beans	Raspberries
Runner Beans	Slowberries	Red Pepper
Slowberries	Spindle	Runner Beans
Spindle	Strawberry	Slowberries
Spring Onions	Sugar snap Peas	Spindle
Strawberry	Tulips	Spring Onions
Sugar snap Peas	Thyme	Sugar snap Peas
Thyme	White Potatoes	Tulips
White Potatoes	Wild Garlic	Thyme
Wild Garlic	Wisteria	White Potatoes
Wisteria		Wild Garlic

		Wisteria

APPENDIX 1c - Freeze-dried natural products which produced no ZOI against MRSA either alone or in combination with penicillin G when extract in water, methanol 20% or ethanol 20%

Freeze-dried natural extracted in a solvent of		
Distilled water	Methanol 20%	Ethanol 20%
Agapanthus	Agapanthus	Agapanthus
Aquilegia	Aquilegia	Aquilegia
Alstroemerias	Alstroemerias	Alstroemerias
Aran Lilly	Aran Lilly	Aran Lilly
Ash Rowan	Ash Rowan	Avocado
Avocado	Bluebells	Baby Corn
Baby Corn	Blueberries	Birds Eye Chillies
Birds Eye Chillies	Camelia	Bluebells
Bluebells	Celery	Blueberries
Blueberries	Chrysanthemums	Camelia
Bryony	Dill	Celery
Camelia	Duranta	Chrysanthemums
Celery	Elder berries	Dill
Chrysanthemums	Fresh Aubergine	Dried Apricot
Dill	Fresh Basil	Dried Cranberries
Dried Cranberries	Fresh Coriander	Duranta
Duranta	Fresh curly Parsley	Elder berries
Elder berries	Fresh Spinach	Fresh Aubergine
Fresh Aubergine	Grape	Fresh Basil
Fresh Basil	Helenium	Fresh Coriander
Fresh curly Parsley	Honeydew Melon	Fresh curly Parsley
Fresh Spinach	Kiwi	Fresh Spinach

Grape	Lilacs	Grape
Hawthorn	Mango	Helenium
Helenium	Papaya	Honeydew Melon
Honeydew Melon	Paprika	Jasmine
Kale	Passion Fruit	Kale
Kiwi	Peach	Kiwi
Lilacs	Phlox	Lilacs
Mango	Pineapple	Mango
Papaya	Pineapple Leaves	Papaya
Paprika	Poppy Seeds	Paprika
Passion Fruit	Red Pepper	Passion Fruit
Peach	Runner Beans	Peach
Pear	Slowberries	Pear
Phlox	Spindle	Phlox
Pineapple	Strawberry	Pineapple
Pineapple Leaves	Sugar snap Peas	Pineapple Leaves
Plum	Thyme	Plum
Poppy Seeds	Wild Garlic	Poppy Seeds
Raspberries	Wisteria	Raspberries
Runner Beans		Red Pepper
Slowberries		Runner Beans
Spindle		Slowberries
Spring Onions		Spindle
Strawberry		Spring Onions
Sugar snap Peas		Strawberry
Thyme		Sugar snap Peas
White Potatoes		Thyme

Wild Garlic		Wild Garlic
Wisteria		Wisteria

APPENDIX Aa Fresh natural products which produced no ZOI against *E. coli* either alone or in combination with penicillin G when extract in water, methanol 20% or ethanol 20%

Fresh natural extracted in a solvent of		
Distilled water	Methanol 20%	Ethanol 20%
Agapanthus	Agapanthus	Agapanthus
Aquilegia	Aquilegia	Aquilegia
Alstroemerias	Alstroemerias	Alstroemerias
Aran Lilly	Aran Lilly	Aran Lilly
Ash Rowan	Ash Rowan	Ash Rowan
Avocado	Avocado	Avocado
Baby Corn	Baby Corn	Baby Corn
Black Pepper Corns	Birds Eye Chillies	Birds Eye Chillies
Bluebells	Black Pepper Corns	Black Pepper Corns
Blueberries	Bluebells	Bluebells
Breakfast Tea	Blueberries	Blueberries
Bryony	Breakfast Tea	Breakfast Tea
Camelia	Bryony	Bryony
Camomile Tea	Camelia	Camelia
Celery	Camomile Tea	Camomile Tea
Chrysanthemums	Celery	Celery
Coffee Beans	Chrysanthemums	Chrysanthemums
Cumin	Coffee Beans	Coffee Beans
Dandelion	Cumin	Cumin

Dill	Dandelion	Dandelion
Dried Apricot	Dill	Dill
Dried Oregano	Dried Apricot	Dried Apricot
Dried Chives	Dried Cranberries	Dried Cranberries
Dried curry leaves	Dried Oregano	Dried Oregano
Dried Marjoram	Dried Chives	Dried Chives
Dried Sage	Dried curry leaves	Dried curry leaves
Dried Tarragon	Dried Marjoram	Dried Marjoram
Duranta	Dried Sage	Dried Sage
Echinacea tea	Dried Tarragon	Dried Tarragon
Elder berries	Duranta	Duranta
Fresh Aubergine	Echinacea tea	Echinacea tea
Fresh Basil	Elder berries	Elder berries
Fresh Coriander	Fresh Aubergine	Fresh Aubergine
Fresh curly Parsley	Fresh Basil	Fresh Basil
Fresh Rocket	Fresh Coriander	Fresh Coriander
Fresh Spinach	Fresh curly Parsley	Fresh curly Parsley
Fuchsia	Fresh Rocket	Fresh Rocket
Goji Berries	Fresh Spinach	Fresh Spinach
Grape	Fuchsia	Fuchsia
Ground Cloves	Goji Berries	Goji Berries
Hawthorn	Grape	Grape
Helenium	Ground Cloves	Ground Cloves
Honeydew Melon	Hawthorn	Hawthorn
Jasmine	Helenium	Helenium
Kale	Honeydew Melon	Honeydew Melon
Kiwi	Jasmine	Jasmine

Lavender	Kale	Kale
Lilacs	Kiwi	Kiwi
Mango	Lavender	Lavender
Marigold	Lilacs	Lilacs
Olive Leaf	Mango	Mango
Papaya	Marigold	Marigold
Paprika	Olive Leaf	Olive Leaf
Passion Fruit	Papaya	Papaya
Peach	Paprika	Paprika
Pear	Passion Fruit	Passion Fruit
Peppermint Tea	Peach	Peach
Phlox	Pear	Pear
Pineapple	Peppermint Tea	Peppermint Tea
Pineapple Leaves	Phlox	Phlox
Plum	Pineapple	Pineapple
Poppy Seeds	Pineapple Leaves	Pineapple Leaves
Raspberries	Plum	Plum
Red Pepper	Poppy Seeds	Poppy Seeds
Runner Beans	Raspberries	Raspberries
Slowberries	Red Pepper	Red Pepper
Spindle	Raspberry Tea	Raspberry Tea
Spring Onions	Runner Beans	Runner Beans
Strawberry	Slowberries	Slowberries
Sugar snap Peas	Spindle	Spindle
Tulips	Spring Onions	Spring Onions
Thyme	Strawberry	Strawberry
White Potatoes	Sugar snap Peas	Sugar snap Peas

Wild Garlic	Tulips	Tulips
Wisteria	Thyme	Thyme
	White Potatoes	White Potatoes
	Wild Garlic	Wild Garlic
	Wisteria	Wisteria

APPENDIX 2b Frozen natural products which produced no ZOI against *E. coli* either alone or in combination with penicillin G when extract in water, methanol 20% or ethanol 20%

Frozen natural extracted in a solvent of		
Distilled water	Methanol 20%	Ethanol 20%
Agapanthus	Agapanthus	Agapanthus
Aquilegia	Aquilegia	Aquilegia
Alstroemerias	Alstroemerias	Alstroemerias
Aran Lilly	Aran Lilly	Aran Lilly
Ash Rowan	Ash Rowan	Ash Rowan
Avocado	Avocado	Avocado
Baby Corn	Baby Corn	Baby Corn
Birds Eye Chillies	Birds Eye Chillies	Birds Eye Chillies
Bluebells	Bluebells	Bluebells
Blueberries	Blueberries	Blueberries
Bryony	Bryony	Bryony
Camelia	Camelia	Camelia
Celery	Celery	Celery
Chrysanthemums	Chrysanthemums	Chrysanthemums
Cumin	Cumin	Cumin
Dandelion	Dandelion	Dandelion
Dill	Dill	Dill
Duranta	Dried Apricot	Dried Apricot
Elder berries	Dried Cranberries	Dried Cranberries
Fresh Aubergine	Duranta	Duranta
Fresh Basil	Elder berries	Elder berries
Fresh Coriander	Fresh Aubergine	Fresh Aubergine

Fresh curly Parsley	Fresh Basil	Fresh Basil
Fresh Rocket	Fresh Coriander	Fresh Coriander
Fresh Spinach	Fresh curly Parsley	Fresh curly Parsley
Fuchsia	Fresh Rocket	Fresh Rocket
Grape	Fresh Spinach	Fresh Spinach
Hawthorn	Fuchsia	Fuchsia
Helenium	Grape	Grape
Honeydew Melon	Hawthorn	Hawthorn
Jasmine	Helenium	Helenium
Kale	Honeydew Melon	Honeydew Melon
Kiwi	Jasmine	Jasmine
Lilacs	Kale	Kale
Mango	Kiwi	Kiwi
Marigold	Lilacs	Lilacs
Papaya	Mango	Mango
Passion Fruit	Marigold	Marigold
Peach	Papaya	Papaya
Pear	Passion Fruit	Passion Fruit
Phlox	Peach	Peach
Pineapple	Pear	Pear
Pineapple Leaves	Phlox	Phlox
Plum	Pineapple	Pineapple
Raspberries	Pineapple Leaves	Pineapple Leaves
Red Pepper	Plum	Plum
Runner Beans	Raspberries	Raspberries
Slowberries	Red Pepper	Red Pepper
Spindle	Runner Beans	Runner Beans

Spring Onions	Slowberries	Slowberries
Strawberry	Spindle	Spindle
Sugar snap Peas	Spring Onions	Spring Onions
Thyme	Strawberry	Strawberry
White Potatoes	Sugar snap Peas	Sugar snap Peas
Wild Garlic	Tulips	Tulips
Wisteria	Thyme	Thyme
	White Potatoes	White Potatoes
	Wild Garlic	Wild Garlic
	Wisteria	Wisteria

APPENDIX 2c: Freeze-dried natural products which produced no ZOI against *E. coli* either alone or in combination with penicillin G when extract in water, methanol 20% or ethanol 20%

Freeze-dried natural extracted in a solvent of		
Distilled water	Methanol 20%	Ethanol 20%
Agapanthus	Agapanthus	Agapanthus
Aquilegia	Aquilegia	Aquilegia
Alstroemerias	Alstroemerias	Alstroemerias
Aran Lilly	Aran Lilly	Aran Lilly
Ash Rowan	Ash Rowan	Ash Rowan
Avocado	Avocado	Avocado
Baby Corn	Baby Corn	Birds Eye Chillies
Bluebells	Birds Eye Chillies	Bluebells
Blueberries	Bluebells	Blueberries
Bryony	Bryony	Bryony
Camelia	Camelia	Camelia
Chrysanthemums	Celery	Celery
Cumin	Chrysanthemums	Chrysanthemums
Dandelion	Cumin	Cumin
Dill	Dandelion	Dandelion
Dried Apricot	Dill	Dill
Dried Cranberries	Dried Apricot	Dried Apricot
Duranta	Dried Cranberries	Dried Cranberries
Elder berries	Duranta	Duranta
Fresh Aubergine	Elder berries	Elder berries
Fresh Basil	Fresh Aubergine	Fresh Aubergine
Fresh curly Parsley	Fresh Basil	Fresh Basil

Fresh Rocket	Fresh Coriander	Fresh Coriander
Fresh Spinach	Fresh curly Parsley	Fresh curly Parsley
Fuchsia	Fresh Rocket	Fresh Rocket
Grape	Fresh Spinach	Fresh Spinach
Hawthorn	Fuchsia	Fuchsia
Helenium	Hawthorn	Grape
Honeydew Melon	Helenium	Hawthorn
Kale	Jasmine	Helenium
Kiwi	Kale	Honeydew Melon
Lilacs	Kiwi	Jasmine
Mango	Lilacs	Kale
Marigold	Mango	Kiwi
Papaya	Marigold	Lilacs
Passion Fruit	Papaya	Mango
Peach	Passion Fruit	Marigold
Pear	Peach	Papaya
Phlox	Pear	Passion Fruit
Pineapple	Phlox	Peach
Pineapple Leaves	Pineapple	Pear
Plum	Pineapple Leaves	Phlox
Raspberries	Plum	Pineapple
Red Pepper	Poppy Seeds	Plum
Runner Beans	Raspberries	Raspberries
Slowberries	Red Pepper	Red Pepper
Spindle	Runner Beans	Runner Beans
Strawberry	Slowberries	Slowberries
Sugar snap Peas	Spindle	Spindle

Thyme	Spring Onions	Spring Onions
White Potatoes	Strawberry	Strawberry
Wild Garlic	Sugar snap Peas	Sugar snap Peas
Wisteria	Tulips	Tulips
	Thyme	Thyme
	Wild Garlic	Wild Garlic
	Wisteria	Wisteria

APPENDIX 4a: Products with an MIC greater than 5.0% when tested against MRSA**.**

Fresh Products + water	**Fresh Products + Methanol**	**Fresh Products + Ethanol**
Curry Leaves	Birds eye Chillies	Aubergine
Dried Cranberries	Baby Corn	Celery
Dried Chives	kiwi	Birds eye Chillies
Dried Tarragon	Pear	Grape
Fresh Basil	Duranta	Paprika
Fresh curly Parsley	Strawberry	Parsley
Fresh Spinach		Pineapple
Spring Onions		Plum
Strawberry		Poppy Seeds
		Raspberry Tea
		Sugar snap Peas
		Tarragon
		Hawthorn
Frozen products +Water Ash Rowan	**Frozen products + methanol** Alstroemerias	**Frozen products + Ethanol** Alstroemerias
Bryony	Birds eye chillies	Bryony
Dried Cranberries	Dried Cranberries	Dried Cranberries
Duranta	Peach	Pineapple Leaves
Red Pepper	Plum	Parsley
	Hawthorn	Strawberry
	Jasmin	Jasmin
Freeze-dried Products + Water	**freeze-dried Products + Methanol**	**freeze-dried Products + Ethanol**
Elderberry	Baby Corn	Rocket
Fuchsia	Birds eye chillies	Fuchsia
Jasmin	Hawthorn	potato
Red Pepper	Cranberries	Tulip
Rocket	Kale	Bryony
	Pear	Hawthorn
	Plum	
	Potato	
	Spring Onion	
	Jasmin	
	Fuchsia	
	Tulip	
	Bryony	