The antibacterial effect of *Berberis vulgaris* mother tincture on *Escherichia coli in-vitro*

A research dissertation submitted to the

Faculty of Health Sciences, University of Johannesburg,

As partial fulfilment for the admission to a

Master's Degree in Technology: Homeopathy

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DECLARATION

I declare that this research dissertation is my own, unaided work. It is being submitted for the degree in Master in Technology at the University of Johannesburg, Department of Complementary Medicine, Faculty of Health Sciences. It has not been submitted previously to this or any other institution for the purpose of obtaining a qualification.

The 25 day of June 2021

ABSTRACT

Escherichia coli (E. coli) is the most abundant facultative anaerobe present in the intestine of humans and many other warm-blooded species. Most strains of *E. coli* are non-pathogenic, co-existing in harmony with their hosts; however, this species can also be regarded as a pathogen capable of causing a wide variety of illnesses. The gastrointestinal tract, meninges and kidneys are among the target organs affected by *E. coli* and diseases resulting from these infections include diarrhoea, sepsis, dysentery, meningitis and even pneumonia. *E. coli's* resistance to major antibiotics is escalating worldwide, highlighting the need for the development of new treatment options. *Berberis vulgaris* L. *(B. vulgaris* L.) has long been used as a herbal remedy in several traditional medicine systems for the treatment of a variety of complaints.

The aim of this study is to determine the antibacterial activity of *B. vulgaris* mother tincture (Ø) on *E. coli* in-vitro, using Kirby-Bauer Disc Diffusion and the Microdilution methods to confirm the minimum inhibitory concentrations (MICs). This quantitative *in-vitro* control study was conducted at the University of Johannesburg Doornfontein Campus at the Water and Health Research Centre (WHRC) with permission granted. The antimicrobial activity of *B. vulgaris* Ø was tested against 32 *E. coli* strains using the Kirby-Bauer Disc Diffusion method (to confirm growth inhibition) and the 96 well dilutions method (MIC). Results were statistically analysed by the researcher with the assistance of a statistician (STATKON). Parametric tests included the Kolmogorov-Smirnova test of normality and one sample T-test. Non-parametric testing comprised of the Friedman test and Wilcoxon Signed Ranks Test to summarize all the data acquired from the susceptibility test procedures.

The results demonstrated that *Berberis vulgaris* Ø inhibited the growth of 90% of the 30 *E. coli* strains tested, which was confirmed by the Kirby-Bauer Disc Diffusion and Microdilution methods. The *B. vulgaris* Ø produced average antimicrobial effects during the Kirby-Bauer Disc Diffusion method, and the minimum dosing or decreased concentration of the active compound may have been an influence. When the *E. coli* bacterial strains were forced into contact with the compound during the microdilution method, growth inhibition was increased. *Berberis vulgaris* Ø has positively demonstrated its clinical efficacy, tolerability and *in-vitro* activity against the pathogenic *E. coli* strains in its entirety.

The outcome of the study displayed that *B. vulgaris* as a mother tincture preparation inhibited the growth of various strains of the *E. coli* bacterial species revealing its potential therapeutic and antimicrobial properties. Further evaluation of this homeopathic tincture as a possible safe and effective alternative antimicrobial agent is essential, and this study has opened up further avenues of research in the field as very limited studies were done thus far.

DEDICATION

My humble effort I dedicate to

To my husband, my best friend

I could never have done this without you and your faith, love, support, patience and constant encouragement

and

for making everything possible….

To my little angel

For showering me with so much love and giving me those moments to never give up

To my parents

Whose love, wisdom, reassurance and prayers day and night got me such a success and honour

and

for raising me to believe that anything was possible….

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Above all, thanks to the merciful Almighty for granting me the ability and strength and lighting up the lamp of hope, courage and patience to the success of this undertaking to the finish. I would like to extend my heartfelt gratitude to all the persons who have contributed endless support, efforts, love and understanding to make my endeavour a reality.

- My sincere appreciation to my supervisor Dr J Pellow for her continued support, aid, encouragement and motivation throughout this process. Thank you for always inspiring, believing and going beyond requirements to make this a success.
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- My parents for their unconditional support, love, faith and encouragement. Thank you for teaching me to believe in myself and my dreams.
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- My beloved husband, M. Ebrahim Mookadam who is always by my side. My deepest thanks and appreciation to you for the endless support, motivation and love throughout this entire journey.
- My loving daughter, Imaan who served as my light and inspiration to pursue this undertaking.

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CHAPTER ONE

1.INTRODUCTION

1.1 Problem Statement

There are over trillions of bacteria present in the human body. Most of these microbes are non-pathogenic while others may be potentially harmful. Enteric bacterial pathogens are the leading cause of infectious diseases and ailments in developing countries, and are generally spread through the faecal-oral route (Gerba, 2014).

Amongst the numerous microorganisms on the surface of the earth, *Escherichia coli (E. coli)* possesses the record of being the highest disease-causing organism. *E. coli* are found in food, our surroundings and the intestinal system of both animals and humans (Lupindu, 2016). This microbe is the most common cause of uncomplicated and community-acquired genitourinary and gastrointestinal tract infections and the foremost reason for the prescription of antibacterial drugs. There are several different strains of *E. coli*; *E. coli* O157:H7 is one of the most pathogenic, and is a Shiga toxin producing strain (Ameer *et al.,* 2020). Excessive use of antibiotics contributes to the development of antibiotic resistance; this in turn, makes treatment of infections difficult and therefore a rapidly growing problem (WHO, 2015). Antibiotic resistance is enhanced and increased when they are incorrectly prescribed and poorly managed (Friedman *et al.,* 2016).

The World Health Organization (WHO) approximates that about 60-75% of the population rely on herbal medications for their primary health care. Many of these herbs have been evaluated for their antibacterial activity and may be utilised to treat an array of diseases and ailments of both parasitic and microbial origin (Gupta, 2017). *Berberis vulgaris L.* (*B. vulgaris L.*) has long been used as a herbal remedy in several traditional systems of medicine, and shows potential benefit as an antimicrobial agent; further research is however needed for a better understanding of its effects on *E. coli* (Madiseh, 2017).

1.2 Aim of the study

The aim of this study was to determine the antibacterial activity of *Berberis vulgaris* mother tincture (Ø) on *E. coli in-vitro*, by means of the Kirby-Bauer Disk Diffusion method and the Minimum inhibitory concentrations (MICs) method.

1.2 Objectives of the study

To evaluate the antibacterial properties of *B. vulgaris* Ø on *E. coli in-vitro* by means of the Kirby-Bauer Disk Diffusion method and MIC methods.

1.3 Hypothesis

It was hypothesized that *Berberis vulgaris* mother tincture would display antibacterial properties against *E. coli in-vitro* by revealing clear zones of inhibition.

1.4 Null Hypothesis

It was hypothesized that *Berberis vulgaris* mother tincture would not display antibacterial properties against *E. coli in-vitro*.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Introduction

Microbes are minuscule living organisms that are present in the water, soil and air, and include bacteria, viruses and fungi. While some microbes are pathogenic and can cause illness, others are necessary for our health and wellbeing (Davis, 2018).

E. coli is a multipurpose bacterium that comprises of harmless as well as pathogenic variants with the capability causing gastrointestinal diseases in both the human and animal species (Leimbach *et al*., 2013). The strains of *E. coli* that typically produce diarrhoeal diseases are identified as the toxigenic *E. coli* and more importantly the Shiga toxin producing *E. coli* (STEC). The *E. coli* O157:H7 strain, in particular, has progressed toward being a major health concern worldwide, as this food-borne pathogen is associated directly with invasive intestinal infections which may be life-threatening. In addition to the consumption of contaminated water and food particles, *E. coli* infections can also be transmitted via person-to-person or person-to animal contact. Those who present with the greatest risk of acquiring infections are the elderly, infants and young children, and those with a debilitated immune system (Gossman *et al*., 2019).

2.2 *Escherichia coli*

E. coli bacteria were initially discovered by a German bacteriologist, Theodor Escherich, in the year 1885. It is typified as being a gram-negative, rod-shaped, facultative bacterium of the class *Escherichia* that belongs to the normal flora of many healthy individuals; it originates or surfaces in foods, beverages, the environment and particularly the lower intestine of warm-blooded organisms (Odonkor & Joseph, 2013).

Most strains of *E. coli* are not detrimental to health and rather act as healthful bacterial gut flora that assist in food digestion. Pathogenic *E. coli* strains are responsible for numerous infectious diseases, including upper respiratory, urinary and gastrointestinal infections, meningitis and septicaemia (Brazier, 2017).

2.2.1 Classification, identification and morphology of *E. coli*

The *E. coli* strains consist of a diverse group of organisms, which are linked by their O-group pattern and virulence gene profiles (Tang *et al.*, 2014). These *E. coli* organisms are categorized into different strains based on their serotypes (Clements *et al.*, 2012). The structure of *E. coli* is prominent, with one circular chromosome and plasmid contained in the genetic material. It possesses the ability to perform complicated metabolic functions to sustain its cell growth and division. The DNA and chromosomal structure of *E. coli* has been entirely sequenced by researchers and displays a variable amount of simplicity (Verma *et al*., 2019).

E. coli bacteria possess coliform and gram-negative characteristics. They contain adhesive fimbriae and a cell wall comprising of an outer lipo-polysaccharide casing, peptidoglycan layer a periplasmic space and an internal cytoplasmic membrane (Baron, 1996) **(Figure 2.1)**.

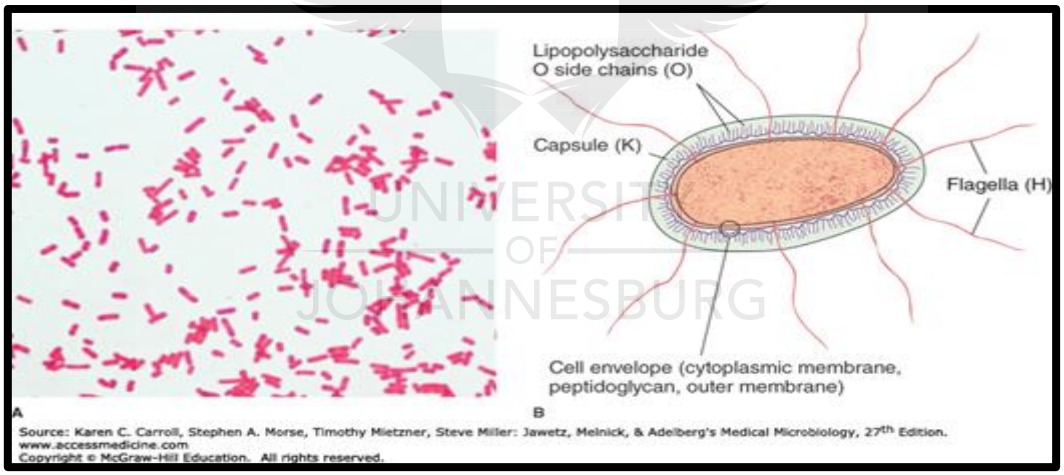


Figure 2.1 Structure and morphology of *E. coli* (Carolli *et al*., Accessmedicine.com, n.d.)

2.2.2 Categories and types of *E. coli*

2.2.2.1 Diarrheagenic E. coli (DEC)

Enteric bacterial pathogens and parasites are the leading cause of communicable diarrhoeal infections that are passed on through the faecal-oral route. Diarrhoeal diseases are responsible

for an estimated three million fatalities each year amid children younger than five years of age (Mokomane *et al*., 2017).

The strains from the *E. coli* category that cause diarrhoeal diseases have evolved to possess a specific set of genetic features which enhance their virulence. Combinations are formed, possessing different integrated genetic factors which thereafter determines the *E. coli* strains that arise and are collectively known as diarrheagenic *E. coli*. (Gomes *et al*., 2016).

The DEC pathogenic types vary according to their host of preference, colonization sites, virulence factors and the subsequent clinical symptoms they produce, and are therefore classified as:

i. Entero-pathogenic *E. coli* (EPEC)
   Initially acknowledged by the serotype, and now clearly known to cause infantile acute diarrhoea. This type of *E. coli* strain has the capability of producing a histopathological lesion in the gastrointestinal epithelium, which is identified as the attaching and effacing (A/E) lesion. Distinctive features of these A/E lesions are the close attachment of the bacteria to the intestinal epithelium on the exterior (Gomes *et al*., 2016; Cepada-Molero *et al*., 2017).

ii. Entero-haemorrhagic (Shiga toxin-producing) *E. coli* (EHEC/STEC)
   EHEC serotype O157:H7 is an *E. coli* strain that mainly causes haemorrhagic diarrhoea and haemolytic uraemic syndrome (HUS). The infections produced by *E. coli* O157:H7 strains range from being asymptomatic to severe (Fatima *et al*., 2019).

iii. Entero-aggregative *E. coli* (EAEC)

   A pathogen accountable for acute and persistent diarrhoea in both adults and kids. Higher morbidity has surfaced in children who are between 0-5 years old and inhabit developing countries (Gomes *et al*., 2016).

iv. Entero-toxigenic *E. coli* (ETEC)
   Enterotoxigenic *E. coli* (ETEC) bacterial types produce heat-labile / heat-stable toxins, and are a major cause of traveller's diarrhoea. ETEC is spread by food or beverages contaminated with human faecal matter (Huang *et al*., 2018).

v. Entero-invasive *E. coli* (EIEC)

EIEC are a collection of intracellular pathogens that enter epithelial cells of the gastrointestinal system, particularly the colon, multiply within and are mobile between adjacent cells; they are therefore the causative agent of bacillary dysentery. Humans infected with EIEC seem to be the key source of infection, as there are no animal reservoirs and transmission is primarily the faecal-oral route (Pasqua *et al*., 2017).

2.2.2.2 Uropathogenic E. coli (UPEC)

It is estimated that an astounding number of individuals worldwide develop a urinary tract infection (UTI) annually, and it is considered the third most common infection present in a hospital environment (Najar *et al*., 2009). This type of pathogenic infection also surfaces and tops the charts with the added costs for the need for numerous hospitalisations and associated high medical expenses (Flores-Mireles *et al*., 2015).

UPEC strains possess an excess of both structural and secreted virulence features which can cause disease. However, the main contributor and determinant of the degree of pathogenicity is the strength to which it attaches to the epithelial cells of the human host at the sites depicted in **Figure 2.2** below (Terlizzi *et al*., 2017).

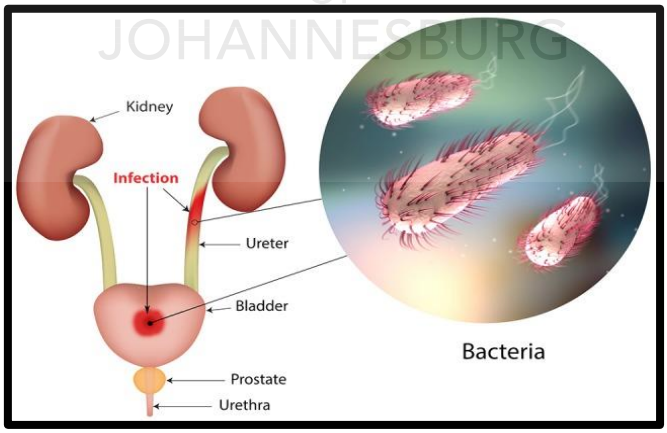


Figure 2.2 The Urinary tract and sites of Infection (Terlizzi *et al*., 2017)

2.3 Infections caused by *E. coli*

Pathogenic *E. coli* strains are responsible for numerous upper respiratory, urinary and gastrointestinal infections, as well as clinical infections such as meningitis (Brazier, 2017).

Besides the consumption of contaminated food and water, *E. coli* infections can be also passed on from person-to-person or person-to-animal contact. Children, the elderly and those with weak immune systems are at an increased risk of contracting an infection (Simon *et al*., 2015).

2.3.1 Urinary tract infections (UTIs)

An infection of the urinary system can include any part of the urinary tract, i.e., the ureters, urethra, kidneys and bladder. The occurrence of UTIs, as seen in **Figure 2.3**, is significantly higher in female patients when compared to males in almost every age group (Tan & Chlebicki, 2016).

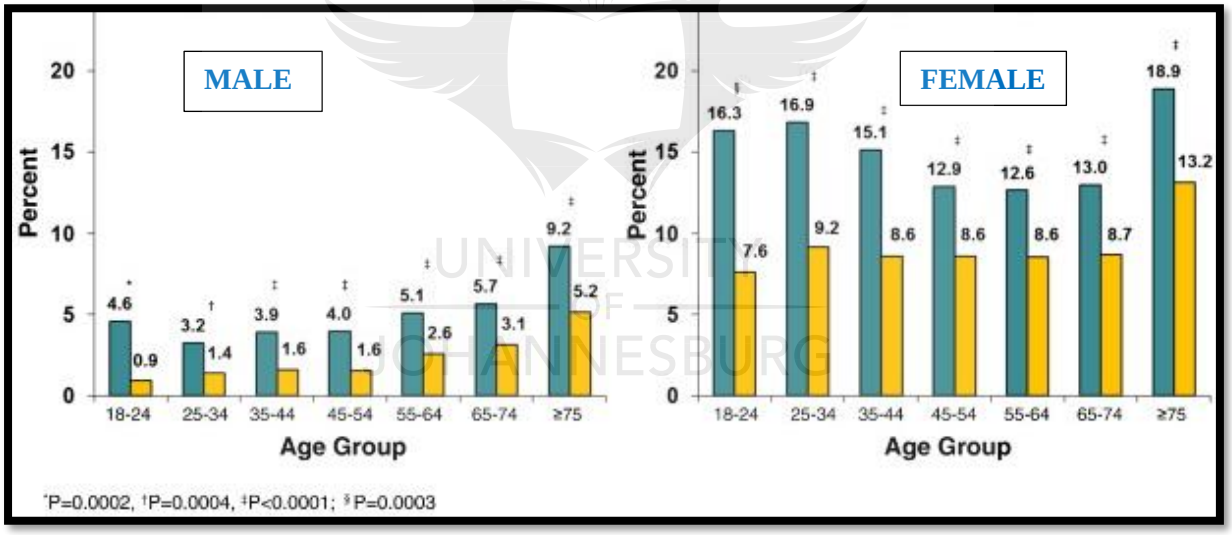


Figure 2.3 Prevalence of Urinary Tract Infections in Primary Care (Fu *et al*., 2014)

According to Storme *et al*. (2019), individuals that have a higher risk of acquiring a UTI are:

- pre/postmenopausal women;
- diabetic patients;
- the elderly;
- children; and

- pregnant women.

Other risk factors include: sexual intercourse, contraception, antibiotic treatment, restrictions such as neuropathic bladder or any mechanical or functional obstruction (Najar *et al*., 2009).

The five stages of a UTI are illustrated in **Figure 2.4**.

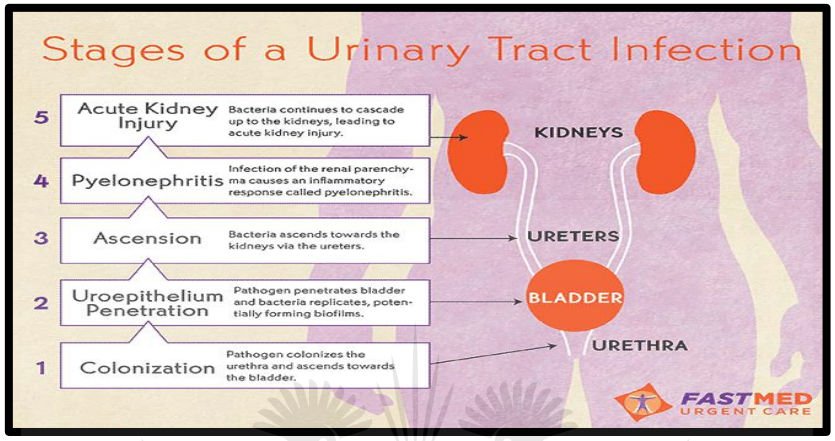


Figure 2.4 The Stages of a Urinary Tract Infection (Gbenga, n.d.)

A UTI begins once uropathogenic *E. coli* (UPEC) enter, attach and ascend the urethra; it thereafter reaches the bladder and binds to the epithelial cells (McLellan & Hunstad, 2016). A subdivision of adherent bacteria is internalized within the kidney/bladder, cytokines and inflammatory response factors are then released, and UTI symptoms begin to surface. These can include:

- foul-smelling urine;
- nausea and/vomiting;
- burning during urination;
- painful and frequent urination;
- increased urgency to pass urine;
- lethargy;
- pain in the back at the kidney region;
- red, milky or clouded urine; and
- a sensation of fullness in the bladder and rectum (Tan & Chlebicki, 2016).

A UTI is clinically diagnosed by means of presenting symptoms and testing of the urine; this can include a urine dipstick and bacterial culture and microscopy (Schmiemann *et al*., 2010). Imaging tests if needed include a computed tomography (CT scan), an ultrasound, intravenous urography and/or an x-ray of the abdomen (Najar *et al*., 2009).

2.3.2 Diarrhoeal diseases

Enteric infections and diarrheal diseases (EIDD) are universal health problems related to poor hygiene and contaminated water supplies, which is a persistent dilemma in developing countries. Diarrhoea is triggered by an extensive array of microbial mediators which belong to viral, bacterial and parasitic categories. Amongst the bacteria, *E. coli* is the leading and most common causative agent. Diarrheagenic *E. coli* (DEC) is accountable for approximately 30-40% of acute diarrhoea in children under the age of five and is significant in both sporadic cases and diarrhoeal outbreaks globally (Saka *et al*., 2019). The Shiga toxin-producing *E. coli* (STEC) and toxigenic *E. coli* are the two main strains that are responsible for causing diarrhoeal diseases. The *E. coli* O157:H7 strain in particular has become a major worldwide food-borne pathogen linked directly to invasive intestinal infections and is also known to result in life-threatening infections (Gossman *et al.*, 2019).

Kapwata *et al*. (2018) recognized the predisposing factors attributed to acquiring diarrhoeal diseases to include:

- inadequate availability of clean water;
- non-existent or unreliable supply of piped water;
- poor hygiene and sanitation practices; and
- poverty.

The symptomology of an *E. coli* infection includes abdominal pain, diarrhoea, haematochezia, foul-smelling and dark coloured urine, lethargy, anorexia and vomiting. These indicative symptoms remain for up to ten days at most; however, are usually not a serious health risk (Gossman *et al.,* 2019).

Clinical diagnosis is based on presenting symptoms and faecal occult blood testing, stool culture, and multiple polymerase chain reaction (PCR) (Barr & Smith, 2014).

2.3.3 Meningitis

Gram-negative bacillary meningitis is a significant cause for increased mortality rates amongst infants worldwide. Regardless of many developments in the management, support and treatment of neonatal meningitis, the fatality rate is between 20-40% (Kim, 2016).

The greatest number of *E. coli* meningitis cases are blood-borne and this bacterium is able to cross the blood-brain barrier. K1 capsular polysaccharide *E. coli* strains, displayed in **Figure 2.5**, are a major source of *E. coli* meningitis during the neonatal period (Bachir& Abouni, 2015).

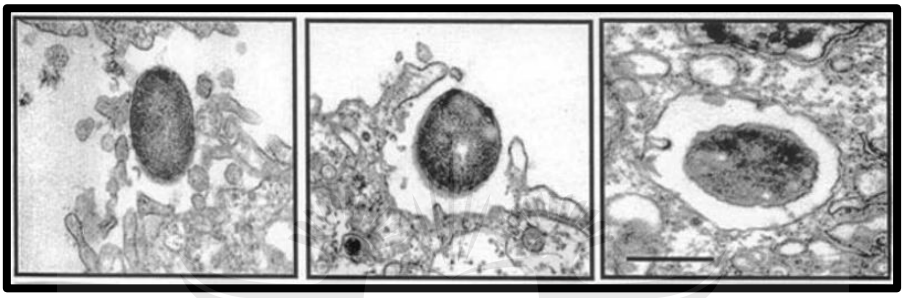


Figure 2.5 Transmission electron micrographs of human brain endothelial cells infected with *E. coli* strain K1 causing meningitis (Kim,2003)

The bacteriological type of meningitis is characterised by inflammation of the meninges in the brain resulting in the release of cytokines/chemokines, as well as penetration of white blood cells and loss of function of the blood–brain barrier (Kim, 2003).

Neonates are particularly predisposed to this type of disease owing to their immature immune system. Meningitis in neonates is problematic to diagnose as the symptomology is very ambiguous. A fever is the clear red flag; though, it does arise at times together with a host of numerous other complaints, including irritability and a decreased appetite (Bundy, *et al*., 2019).

The clinical signs of neonatal meningitis, according to Ku *et al*. (2016), can be subtle and nonspecific:

- vomiting
- apnoea

- hypo/hyperglycaemia
- bradycardia
- perfusion
- seizures
- hypotonia
- bulging of the anterior fontanel
- jaundice and /or
- diarrhoea.

The diagnosis of meningitis is confirmed with a lumbar puncture and culturing of the cerebrospinal fluid (CSF) to identify the causative organism (Ku *et al*., 2016). The polymerase chain reaction (PCR) has also been utilised as a diagnostic means for testing meningitis and exhibits an enhanced sensitivity and specificity. The PCR testing method permits faster detection of pathogens when equated with the traditional cultures (Bundy *et al*.,2019). It is very difficult to predict the diagnosis of meningitis exclusively on the CSF restrictions; this thereby signifies that the culture of CSF remains the gold standard for diagnosis (Smith, 2015).

In order to progress and expand on therapeutic results in infants diagnosed with meningitis, numerous adjunctive treatments have also been employed, as well as the use of intraventricular antibiotics such as ampicillin and gentamicin, oral glycerol immunoglobulins and granulocyte macrophage colony-stimulating factor (Ku *et al*., 2016).

2.4 Conventional treatment for infections caused by *E. coli*

Antibiotic treatment is widely utilised against bacterial infections. It is defined as pharmacological agents that prevent or destroy the bacteria, with rarely any noticeable effect on the host (see **Figure 2.6**). Antibiotics that are bacteriostatic avert further duplication of bacteria and consequently depend on the immune system to remove the infection, however, bactericidal antibiotics destroy and get entirely rid of the bacteria (Baquero *et al*., 2020).

Antibiotics are the main course of treatment for *E. coli* infections and typically include amoxicillin, ampicillin, semisynthetic penicillin, gentamicin, cephalosporins-cefepime

and imipenem, ciprofloxacin and the aminoglycosides. Excessive use of antibiotics and antimicrobial drugs, especially in situations when it is not warranted, contributes to the development of resistance, therefore making treatment of infections difficult (Sabina, 2016).

E. coli multi-drug resistance (MDR) is of growing concern due to the rapid increase in the number strains of *E. coli* resistant to first-line antibacterial drugs such as ampicillin, trimethoprim-sulfamethoxazole and gentamicin (Sabina, 2016).

For diarrhoeal infections caused by Shiga toxin-producing *E. coli*, antibiotics are not usually recommended, as they essentially upsurge the amount of Shiga toxin in the system and can worsen the symptomology (Humphries *et al*., 2015).

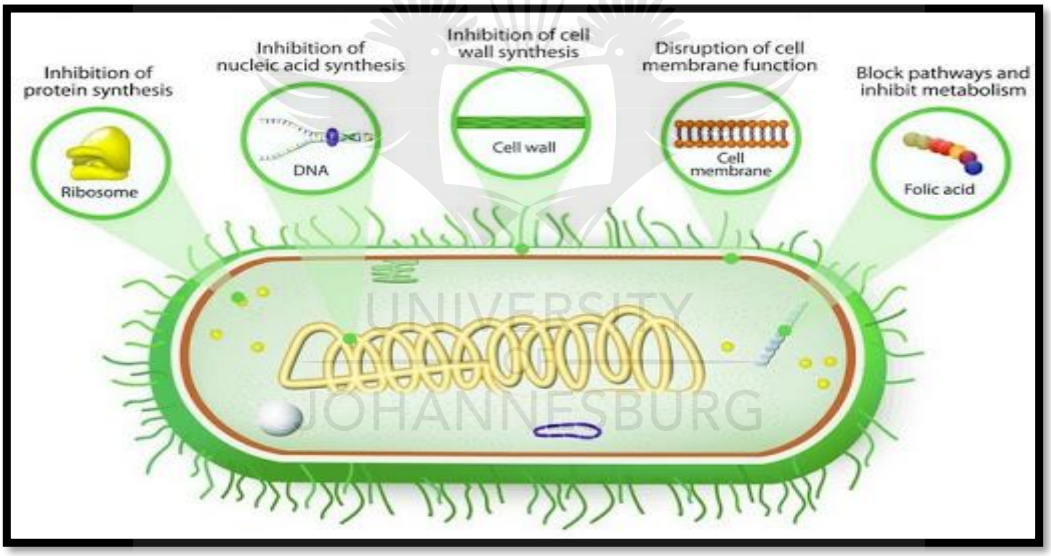


Figure 2.6 Mechanism of action for antibiotics (Saxena, 2021)

2.4.1 Types of antibiotics for *E. coli* infections

2.4.1.1 Amoxicillin

This is one of the most utilised antibiotics in primary care and for neonates. Amoxicillin is known as an amino-penicillin as it is formed by the addition of an amino group to penicillin. Amoxicillin is placed in the beta-lactam category of antibiotics, and treats various gram-positive and gram-negative bacteria (Tang *et al*., 2019). It produces minimal side effects and

is tolerated well by most individuals, however complaints of the gastrointestinal system, such as vomiting and diarrhoea, may occur. Crystalluria, nephritis, and haemolytic anaemia have also been reported with prolonged administration. Another significant complication to be aware of is hypersensitivity reactions (Akhavan *et al*., 2020).

2.4.1.2 Cefepime

Cefepime is a parenteral fourth-generation cephalosporin with an extensive range of antimicrobial activity. It is usually prescribed for an array of infections developed at hospital and clinics; however, it is well endured and only associated with a rare number of adverse reactions (Mac *et al.*, 2015). Cefepime is a mediator that acts by inhibiting the synthesis of the bacterial cell wall. It acts on both gram-positive and gram-negative microbes (Shah *et al.*, 2016).

2.4.1.3 Imipenem

Imipenem, which falls under the carbapenems category, is a powerful antibiotic with relatively few adverse effects. This type of drug is often utilised extensively as an effective antimicrobial agent for hospitalized patients in need of intensive care (Baughman, 2009).

Carbapenems disrupt the microbes' cell wall development and structure, and covers anaerobic and gram-negative bacteria. The most common adverse effects are difficulties at the infusion site and disruption of the digestive system (Pap-Wallace *et al*., 2011; Nicolau, 2008).

2.4.1.4 Ampicillin

Ampicillin is presented as an extended-spectrum penicillin and is active against gram-positive and gram-negative microbes. Ampicillin has an operative minimum inhibitory concentration (MIC) of 4 mg/L for many pathologically significant bacteria present in infectious diseases (Kaushik *et al*., 2014).

It is administered intramuscularly as an alternative to penicillin, orally and even intravenously. This antibiotic has FDA approval for treating infections such as:

- septicaemia;
- endocarditis;

- respiratory tract infections;
- meningitis;
- genitourinary infections; and
- gastrointestinal infections (Peechakara *et al., 2020)*.

The adverse effects displayed by ampicillin comprise mainly of allergic skin reactions, seizures, pseudomembranous colitis, vomiting, drug-induced diarrhoea, haemolytic anaemia and pneumonia. Ampicillin is contraindicated in patients with hypersensitivity reactions and infectious mononucleosis (Hamao *et al*., 2020).

2.4.1.5 Penicillin

In the year 1960, the third generation and broad-spectrum penicillins called aminopenicillins were brought to light. The discovery and production of penicillin has saved millions of lives over the last century (Gaynes, 2017). Amoxicillin and ampicillin are key examples of this category and has demonstrated their undoubted effectiveness against a wide-range of gram-negative bacteria (Fair, 2014).

The most common side effects include: headaches, skin rash, hives, nausea and diarrhoea. Less commonly, abdominal cramps, fungal infections, joint pain and shortness of breath may occur. Although the use of penicillin is widespread, some safety concerns can occur. Individuals who are breast-feeding might pass slight amounts of penicillin to the infant. This can result in the child experiencing allergic reactions, diarrhoea, fungal infections, and a skin rash. Individuals with kidney disease have an increased risk of side effects, and patients with a history of stomach ulcers or other intestinal diseases might be more likely to develop colitis when taking penicillin (Newman, 2018).

2.4.1.6 Gentamicin

It is an aminoglycoside antibiotic that was among the first to be utilised in medical practice globally due to its accessibility, affordability and excellent efficacy as an antimicrobial.

Gentamicin is poorly absorbed in the gastrointestinal tract and therefore must be administered parenterally or topically (Chavez *et al*., 2021).

These antibiotics are usually used to treat infections created by gram-negative bacteria and work synergistically with other antibiotics. Aminoglycosides vary in their capability to cause vestibular and cochlear toxicity, and gentamicin and streptomycin may cause bilateral vestibulopathy, and in high dosages hearing loss may occur (Hain *et al*., 2018; Kushner *et al*, 2017).

2.4.1.7 Ciprofloxacin

Ciprofloxacin is a second-generation fluoroquinolone and is active against mycobacteria, gram-positive and gram-negative bacteria and anaerobes. Advances in the pharmacokinetics and pharmacodynamics of quinolones have led to the betterment in metabolism, removal and transport, leading to enhanced antibiotic dosing which boosts the effectiveness and prevents resistant mutations (Ogbru, 2019).

Ciprofloxacin is utilised for many forms of infections namely UTIs, prostatitis, typhoid fever and otitis media. Adverse drug reactions of this antibiotic include diarrhoea, abdominal cramping, nausea and vomiting. Rare reactions that may occur are hearing impairment and phototoxicity (Reis *et al*., 2016).

2.5 Antibiotic resistance

Antibiotic resistance is an evolving universal problem in both human and veterinary medical science. The extensive practice of antibiotics in medicine and agriculture is the key driving force behind the high prevalence of antibiotic resistance (Rasheed *et al*., 2014). The global emergence of multidrug-resistant gram-negative bacteria is an escalating threat (Li *et al*., 2015), as infections remain the leading cause of death in the developing world (Kapoor *et al*., 2017)

Antibiotic resistance arises when the bacterial cells are altered with the utilization of these drugs (CDC, 2020). An increase in hospitalisations, medical care bills and mortality rates are indicative of an outcome of major resistance to antibiotic medications (WHO, 2015).

Antimicrobial resistance mechanisms fall into four main categories, as illustrated in **Figure 2.7**:

- Limited uptake of the drug – this occurs when the permeability of the outer membrane is decreased;
- Adjustment of drug target – this occurs by changing the structure and quantity of binding proteins present, altering the DNA through mutations and producing resistance enzymes showing the folate biosynthesis pathway.
- Inactivation of the drug - this occurs through degradation of the antibiotic, or transference of a chemical group to that specific drug.
- Active drug efflux- this occurs with bacteria that hold chromosomally encoded genes for efflux pumps. A few are displayed constitutively while others are induced, from certain stimuli or a suitable substrate. The role of the efflux pump mainly is to clear the cell of toxic elements. The level of competence with regards to resistance is influenced by means of the carbon source present (Kapoor *et al*., 2017).

These mechanisms could be innate to the microbes or attained from other microorganisms. The gram-negative bacteria practice all four of the main mechanisms (Reygaert, 2018). Efflux pump genes and proteins exist in bacteria that are mutually susceptible and resistant to antibiotics. Pumps may be specific for one substrate or may transport a range of structurally dissimilar compounds linked to multiple drug resistance (MDR). MDR efflux pumps contribute to antibiotic resistance through: inherent resistance to a complete class of mediators, characteristic resistance to specific agents and resistance by an efflux pump that was over expressed (Piddock, 2006).

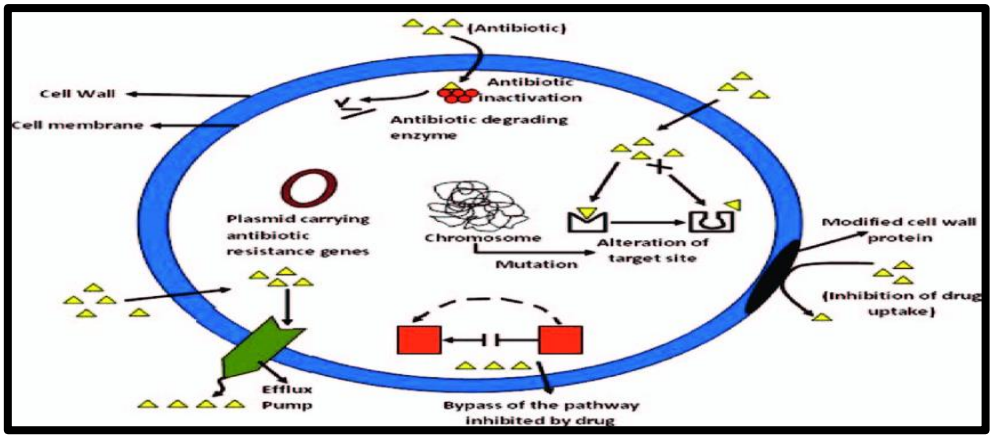


Figure 2.7 Mechanisms of antibiotic resistance (Singh, 2014)

The resistance to antibiotics is escalating and hindering new developments and means in the prevention and treatment of a large number of infectious diseases (WHO, 2018). The growth and spread of resistance to antibiotics are escalated in countries where unprescribed antibiotics can be purchased over-the-counter. Likewise, in countries lacking rules and regulations for standard medical treatment, antibiotics are frequently over-prescribed by medical professionals and excessively used by the public (Tangcharoensathien *et al.* ,2018).

E. coli bacteria have developed a resistance to third-generation cephalosporins and several of the antibiotics named above. A major downfall in this regard is the absence in the development of new classes of antibiotics. When resistance is established in one type of antibiotic, it opens the door to a multi-resistance of drugs in a similar / same category (Utt & Wells, 2016). Changing circumstances worldwide requires altering the way antibiotics are used and prescribed. If a major advance in medication develops that is deprived of any behaviour change, antibiotic resistance and a decreased antibiotic compliance will continue to persist as a major hazard (Ventola, 2015).

2.6 Homeopathy

Homeopathy is a natural form of medicine utilised by over 200 million people globally to treat acute and chronic ailments. The holistic nature of homeopathy denotes that an individual is treated uniquely and their bodily and neurological functions are all taken into consideration for the managing and prevention of disease (Shurtleff, 2018). Taking all these aspects into account,

a homeopathic practitioner will select the most fitting medicine based on the individual's specific symptoms and level of health, to stimulate their own healing ability (Ratini, 2021).

Homeopathy was developed by Dr Samuel Hahnemann in 1796. It utilises treatment that stimulates the body's natural healing mechanisms on all three plains- physical, mental and emotional and is based on four key principles (Das, 2019).

2.6.1 Law of similars

The law of similars is based on the principle of 'like cures like' or *'similia similibus curentur',* a claim that a substance that causes the symptoms of a particular disease in healthy individuals would cure similar symptoms in the ill (Bloch *et al*., 2008).

2.6.2 Law of the minimum dose

A belief that the lesser the dosage of the medicine taken, the greater its effectiveness. Homeopathic remedies undergo potentization (serial dilution and succussion), making the remedy more powerful (Shurtleff, 2018). It includes administering the minimum possible dose to enhance its health-promoting effects and decrease any side effects that may arise. Repetition of the dose of the remedy is decided based on the person's response to the medication.

2.6.3 Law of the single remedy

This law states that a single homeopathic remedy is administered at any one time. It would become problematic to determine the action of multiple homeopathic remedies prescribed to an individual all at once. The foundation of homeopathy is individualised treatment with a single remedy (Schepper, 2000).

2.6.4 Potentization of the remedy

This method occurs through a procedure of a series of consecutive dilutions, wherein a very dilute extract is attained. With every step of dilution that occurs, the remedy is energetically succussed (shaken). This process of succussion is intended to awaken and produce the dynamic nature of the medication that in turn positively affects the vital force (life force) of the individual (Tounier *et al*., 2019).

2.7 *Berberis vulgaris* L. *(B. vulgaris* L.*)*

B. vulgaris L. has played an important role in traditional herbal medicinal systems for more than 2 500 years. Its medicinal properties include antimicrobial, antipyretic, antioxidant, anti-inflammatory and sedative effects (Madiseh *et al.*, 2017). It is a renowned therapeutic plant from the Berberidaceae family, and grows mainly on the continents of Europe, Africa and Asia. The phytochemical research carried out regarding the many species of *Berberis* have resulted in the isolation of specific alkaloids, sterols, tannins and phenolic compounds (Mohammadzadeh *et al*., 2017).

B. vulgaris L. contains alkaloids such as berberine, berbamine, palmatine and many secondary metabolites like ascorbic acid, pectin and tannin. Berberine is an isoquinoline alkaloid with the chemical structure illustrated in **Figure 2.8**. Berberine has been isolated and evaluated as a medicinal application and is the major active component of various plant species, including *B. vulgaris* L. (Dashti, 2014). Current studies have shown that berberine displays numerous pharmacological activities such as:

- antioxidant;
- anti-inflammatory;
- anti-diarrhoeal;
- antimicrobial; and
- anti-tumour activity (Dashti, 2014).

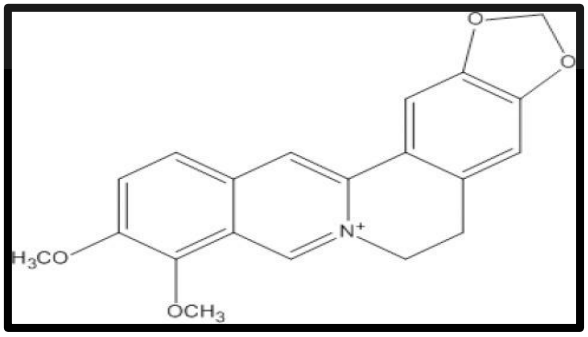


Figure 2.8 Chemical structure of Berberine (Chatuphonprasert, 2013)

Different species of plants that are rich in berberine exhibit various pharmacological and therapeutic actions, for e.g., immunomodulatory effects; protection of the cardiovascular system, liver and kidneys; endothelial relaxation; and antioxidant effects (Neag *et al*., 2018).

In addition, berberine can also be active and protective against natural and chemical toxins. Numerous medical trials up to date, correspondingly presented with protection and prevention properties of berberine against metabolic diseases too (Belwal *et al*.,2020). The defensive effects of these compounds are visible in many organs such as the brain, kidney, heart, liver and lung and have been verified in many studies done thus far (Mohammadzadeh, 2017).

2.7.1 Preparation methods of medicinal plants

2.7.1.1 Herbal tinctures and extracts

Herbal tinctures are concentrated herbal extracts that are prepared according to the methods laid out in herbal pharmacopoeias. Alcohol is considered the best solvent in order for the herbal characteristics to be extracted. Tinctures can be manufactured using the whole plant or parts of the plant, such as fresh/dried flowers, fruits, leaves, roots and bark (Cirino, 2019).

There are two methods for preparing a herbal tincture:

- The percolation method: In this method the material chosen from the plant is dampened preceding their placement into the percolator with an appropriate quantity of the menstruum. It is thereafter left to infuse for a time period of four hours in an airtight container. To extract the maximum residual fluid retained, the mass is thereafter pressed. The final step is to get a suitable proportion which is then filtered by decantation.
- Maceration (folk) method: The maceration method is more commonly used. This process is initiated when the plant material is placed in pieces or powder in a container of menstruum and allowed to stand for 36 to 48 hours. It is thereafter frequently shaken up until extraction of the plant material is fully obtained. The material is then strained and the remains of the solid are pressed to gain all the liquid that remains. Finally, the liquid product is clarified by means of filtration (Azwanida ,2015).

2.7.1.2 Homeopathic mother tinctures

Homeopathic mother tinctures are a solution prepared from substances in the plant or animal kingdom by the procedure of maceration or percolation; this is achieved by means of utilising a suitable menstruum (solvent) in a fixed quantity (German Homeopathic Pharmacopoeia,

2013). The mother tincture is present in a 1:10 dilution and utilised in this form (Handa *et al*., 2008). The typical solvents used for extracts are alcohol, vinegar, or vegetable glycerine. Mother tinctures form the basis for the remedies made in potency (German Homeopathic Pharmacopoeia, 2013).

2.8. *Berberis vulgaris (B. vulgaris)* mother tincture (Ø)

The homeopathic preparation of *B. vulgaris* L. is named *B. vulgaris*. It is homeopathically indicated for conditions affecting the liver, kidneys and bladder (Vermeulen, 2005). A homoeopathic *B. vulgaris* mother tincture is prepared according to the German Homoeopathic Pharmacopoeia Hahnemannian method HAB 4A (Appendix B) (German Homoeopathic Pharmacopoeia, 2013).

The yellow root bark is the most concentrated source of active ingredients and most used in preparation of the tincture; however, all the parts of this plant can be utilised. The alkaloid berberine contained in *B. vulgaris* has established the greatest amount of research and the most widespread approval as the active component. Other constituents are tannins, oxyacanthine, berbamine, wax and resin. Berberine and its associated constituents are antibacterial (Arayne *et al*., 2007).

Figure 2.9 *Berberis vulgaris* L. (European barberry) (Etsy Inc.com, n.d.)

Homeopathically, *B. vulgaris* also known as the European barberry seen in Figure 2.9 above is widely used in the treatment of urolithiasis, to alleviate pain and stone formation (Ganesan, 2015). *B. vulgaris* is an important remedy for neuralgic pain or colic of any part of the body but especially the joints and muscles and the urinary system. Berberine inhibits bacteria from attaching to human cells, which helps prevent infection and therefore a successful antibacterial (Arayne *et al*., 2007). Characteristic sharp, stitching or pains that radiate are caused by cystitis, forms of arthritis and kidney stones, nonetheless these are rapidly ameliorated by this remedy (Vermeulen, 2005). It permits the passing of kidney stones with a considerable reduction of colic.

B. vulgaris is suited for both urinary tract infections and diarrhoeal diseases caused by *E. coli* and other gram positive/negative bacteria. It is indicated for a rapid change of symptoms as characteristic pains change with regard to place and character (Vermeulen, 2005).

2.9 Related research

Currently there is very limited research on the anti-microbial effects of this medicinal plant, and only the herbal preparations have this far been evaluated. A study by Shahid *et al.* (2017) was conducted to determine the *in-vitro* antimicrobial activity of various plant extracts of the fruit of *B. vulgaris* L. against nine clinically isolated pathogenic microbes - *Staphylococcus aureus, Protea mirabilis, Salmonella typhi, Salmonella para-typhi A* & *B, Klebsiella pneumonia, Streptococcus β, E. coli* and *Pseudomonas aeruginosa.* Disc diffusion and dilution methods were used; in the diffusion method, the fruit extract of *B. vulgaris* L. exhibited some antimicrobial activity against *Pseudomonas aeruginosa* only, but in the dilution method, this extract showed antimicrobial impacts against all pathogens tested.

Some research pertains to other related species of *B. vulgaris* L. The antimicrobial action on the root bark of *Berberis lycium* (a different species to *B. vulgaris* L.*)* and its key constituent berberine, was tested against other microbial strains via the agar well diffusion test and thereafter additionally analysed using the micro-broth dilution method. Amongst the bacterial strains present, *E. coli* stood out to be the most vulnerable, while in the fungal category *Candida albicans* displayed its susceptibility to berberine as well as the crude

methanolic extract of the plant. Berberine was more effective than crude extracts for *Candida albicans*. The MIC index of the crude methanolic extract demonstrated a bactericidal effect for *E. coli* bacteria (Malik *et al*., 2017).

According to another study conducted by Singh *et al*. (2007), the antimicrobial activity of hydroalcoholic extracts of four Berberis species - *Berberis aristata, Berberis asiatica, Berberis chitria* and *Berberis lycium* were tested against eleven bacterial and eight fungal strains. The root extract from *B. aristata* resulted in low minimum inhibitory concentration values against *Bacillus cereus, E. coli*, *Staphylococcus aureus* and the *Aspergillus flavus*.

Berberis microphylla (B. microphylla) is a native plant frequently used by indigenous ethnic groups in traditional medicine as an antiseptic. One study evaluated the antibacterial activity of alkaloid extracts of *B. microphylla* leaves, stems and roots, used individually or in a blend with antibiotics against gram positive and negative bacteria. The *in-vitro* anti-bacterial actions of the alkaloid extracts contained presented noteworthy activity, however only against the gram-positive bacteria. Berberine, the primary constituent of the alkaloid extracts, showed positive results only against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Synergistic properties between the alkaloid extracts and antibiotics against the bacterial strains were also confirmed (Manosalva *et al*., 2016).

In a study by Sharma *et al*. (2011), antimicrobial properties of *Berberis aristate (B. aristate)* leaves and root extracts were tested by the agar well diffusion method against six common ear pathogens, namely:

- *Staphylococcus aureus;*
- *Proteus mirabilis;*
- *Escherichia coli;*
- *Pseudomonas aeruginosa;*
- *Acinetobacter spp.; and*
- *Candida albicans*.

The root extracts displayed antimicrobial activity against all six pathogens however the extracts from the leaf exhibited positive results against only five. The maximum antimicrobial activity of *B. aristata* root extract was towards *S. aureus*. This study proposes that the *B. aristata* organic extracts presented an extensive spectrum of antimicrobial activity and may be suited and beneficial in treating ear infections (Sharma *et al*., 2011).

CHAPTER THREE

3. METHODOLOGY

3.1 Research design

This quantitative *in-vitro* control study was conducted at the University of Johannesburg, Doornfontein Campus at the Water and Health Research Centre (WHRC) with permission granted (Appendix A).

3.2 Bacterial strains

E. coli strains stored at the WHRC were used for this study. This included two reference strains (ATCC11775 and ATCC 8739) and 30 strains isolated from previous projects (APPENDIX C). Strains were stored as glycerol stocks at -80°C and were plated onto Mueller-Hinton agar plates and grown overnight at 35°C.

3.3 Homeopathic tincture

The *Berberis vulgaris* Ø was purchased in the homoeopathic mother tincture (Ø) form from a commercial source (Fusion Homeopathics) that prepares the tincture according to the HAB4A (Appendix B) method laid out in the German Homoeopathic Pharmacopoeia (German Homoeopathic Pharmacopoeia, 1993). The mother tincture was used as a test tincture with a final alcohol percentage of 62% and the herbal extract berberine as the active control for the experiments.

Ethanol at 62% can denature the proteins of microbes and therefore enhance their ability to inactivate bacteria and viruses (Jing *et al*., 2020) and this should be taken into consideration when describing the potential effect of the mother tincture prepared in this solvent.

3.4 Method and procedure

Fresh bacterial cultures were prepared for each experiment by streaking the bacteria onto Mueller-Hinton agar plates using sterile cotton swabs and incubated for 24 hours at 35°C. Bacterial suspensions for use in the experiments were prepared with buffered saline and the

cell density adjusted to match 0.5 McFarland standards to ensure comparable results. This suspension was utilised within 30 minutes of the preparation.

3.4.1 Kirby-Bauer disc diffusion method

The Kirby-Bauer test, also known as the disc diffusion method, is the most extensively used susceptibility test in determining what choice of medicine ought to be used when treating an infection. This technique relies on the inhibition of bacterial growth measured under standard conditions. The antimicrobial activity of *B. vulgaris Ø* was determined by the Kirby-Bauer disc diffusion methodology, which is in accordance with the Clinical and Laboratory Standard Institute (Cockerill *et al*., 2012), using the prescribed standardized protocol (Hudzicki, 2013).

A 100µl of each of the *E. coli* strain solutions adjusted to a 0.5 McFarland standard (Section 3.2) was plated onto the Mueller-Hinton agar plates using sterile swabs creating a bacterial lawn and left to dry for approximately 5-10 minutes. Each antimicrobial disc was placed onto the surface of all the agar plates using flame sterilized forceps using the layouts as shown in **Figure 3.1**. The discs were then impregnated with 20µL of the required concentration as illustrated below in **Figure 3.1**.

This experiment was conducted in triplicate, i.e., 3 plates for each strain (90 plates) to ensure repeatability on three independent days (reproducibility) to obtain optimal and reliable results. Appropriate controls were included in the experiments to test the reliability and trustworthiness of the methods, and included a media control (negative control), distilled water (negative control), Imipenem (positive control), Cefepime (positive control) and 62% (v/v) ethanol (solvent control).

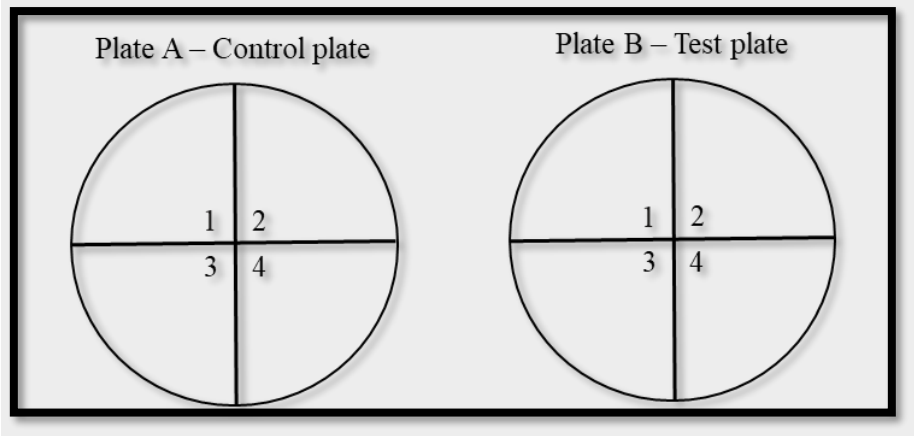


Figure 3.1 Template for the Mueller-Hinton agar plate for the Control plate (A) and Test Plate (B). The layout for plate A is 1) Imipenem (10µg), 2) Cefepime (30µg), 3) distilled water and a blank disc (4). The layout for plate B is 1) *Berberis vulgaris Ø*, 2) Ethanol (62% v/v), 3) distilled water and a blank disc (4).

Inversion of the agar plates were then conducted and placed in a 35˚C incubator for a period of 16-24 hours. Once the plates were incubated, they were examined for a zone of inhibition and measured in millimetres around the impregnated discs by means of a ruler and used to determine if there was inhibition of bacterial growth.

3.4.2 Minimum inhibitory concentration (MIC)

The MIC of the *B. vulgaris* Ø was tested using the serial dilution method in 96 well plates using the layout shown in **Figure 3.2**. Mueller Hinton broth (100µl) was added to each well followed by the mother tincture (50 µl) that was added to the first well, mixed and 50 µl removed, mixed to the next four wells with the 50 µl removed from the sixth well and discarded. The same controls were included as in section 3.4. Plates were covered with a lid and incubated at 35°C for 16-18 hours. After the incubation period, 50 µl Iodonitrotetrazolium chloride (INT) dye was added to the wells and incubated again at a temperature of 35°C for approximately 30 minutes to produce a pink to dark purple colour if the bacterial cells were metabolically active (Eloff, 1998).

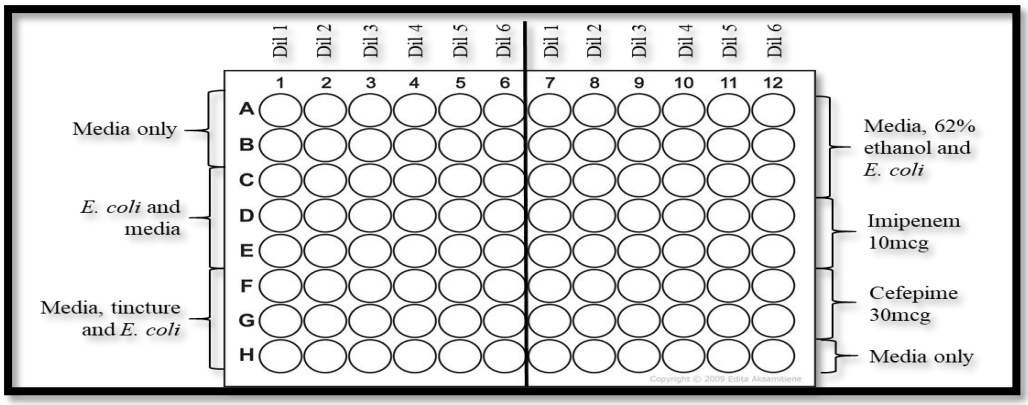


Figure 3.2 Template of the 96 well plate and its contents used to determine the MIC of *B. vulgaris* Ø against the *E. coli* strains.

3.5 McFarland standard

The McFarland Standard is used as a reference to adjust the turbidity of the bacterial suspension contained in the vial. It aids in maintaining the number of bacteria present inside a specified range to standardize the microbial testing. This standard can be prepared in variable concentrations extending from 0.5 to 4. Utilised the most for antimicrobial susceptibility testing is the 0.5 McFarland standard, as was done in this study.

A McFarland Standard is a chemical combination of sulphuric acid together with barium chloride in a solution form. The chemical response amid these two chemicals results in the creation of a barium sulphate precipitate. Following a shake of the resulting solution, the turbidity of a McFarland Standard is now visually analogous to a bacterial suspension of a known concentration. Through merely fine-tuning and adjusting the volume of the two-main biochemical elements, McFarland standards of variable degrees of turbidity can be prepared, which signifies different bacterial densities depicted below in **Figure 3.3**. A standard of 0.5 McFarland turbidity delivers an optical density which is then equivalent to the density of a bacterial suspension with a 1.5 x 10^8 colony forming units (CFU/ml).

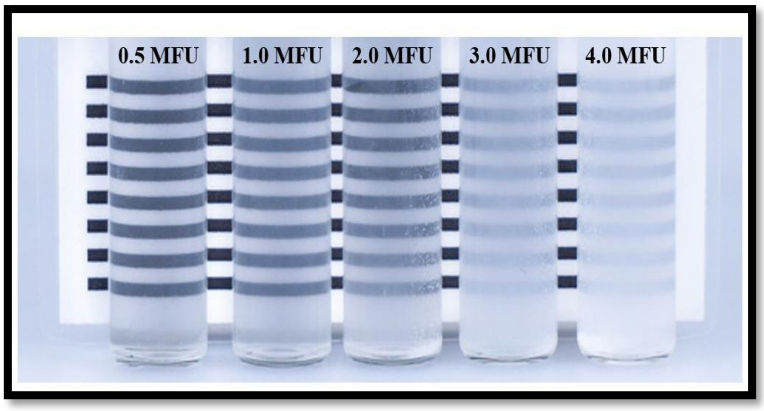


Figure 3.3 McFarland Standard in front of a Wickerham card (Aryal,2020)

3.6 Data collection

The uniform circular area surrounding the antibiotic disc that has no bacterial growth is known as the zone of inhibition. The zones of inhibition around each disc were measured and the results were recorded in millimetres (mm).

For the MIC plates the last well that did not have metabolically active bacterial cells as determined with the INT dye was seen as the highest dilution, or lowest concentration that inhibited the *E. coli* growth.

The concentration of the antibiotic, solvent or tincture in the well was calculated using

Equation 1:

$$C_i \times V_i = C_f \times V_f$$

Where C_i = initial concentration test solution added

V_i = initial volume test solution added

C_f = final concentration of test solution

V_f = final volume in well before you remove the volume for the next well

3.7 Data analysis

The results were captured in Microsoft Excel sheets and analysed by the researcher with the assistance of a statistician at STATKON (Van Staden, 2021) using SPSS version 27.0. The tests included parametric and non-parametric tests. Parametric tests included the Kolmogorov-Smirnova test of normality and one sample T-test. Non-parametric tests included the Friedman test and Wilcoxon Signed Ranks Test. Pearson correlation was also conducted and completed to determine the antimicrobial effects compared to the antibiotic control.

3.8 Ethics

This study was approved by the University of Johannesburg (UJ) Faculty of Health Sciences Research Ethics Committee (REC) and the Higher Degrees Committee (HDC) (Appendices D and E respectively). The researcher was appropriately trained to conduct the experiment and ensure the prevention of contamination or exposure to the *E. coli* strains. All equipment was properly cleaned and sterilized before each use to avoid contamination and compromised results. The safety protocols were always adhered to, to ensure laboratory safety, safe removal and disposal of bacterial organisms, tinctures and equipment.

Protective clothing such as gloves, safety glasses and laboratory coats were worn at all times to maintain and ensure the safety of the researchers and technicians involved. The experiment was conducted under the supervision of trained laboratory personnel and all waste were discarded as biological waste and collected and discarded by the UJ approved waste collector in accordance with the relevant UJ policy.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Introduction

The aim of this study was to investigate the antimicrobial effect of *B. vulgaris* Ø against different strains of *E. coli*. This was attempted using the Kirby-Bauer Disc Diffusion and 96 well plate Minimum Inhibitory Concentration (MICs) tests as discussed in Chapter 3. Due to the positive results, the data attained from the tests was analysed with the assistance of a statistician using parametric and the nonparametric tests to determine the antimicrobial effects of *B. vulgaris* Ø in comparison to the antibiotic control. All of the antibacterial and susceptibility tests were completed with assistance from a qualified laboratory technician at the Water and Health Research Centre at the University of Johannesburg. The decision was taken to present the results, data interpretation and discussion after each appropriate subheading and in one chapter to allow the reader to follow arguments that influence the different sections.

4.2 Antibacterial Experimental Results and Discussion

4.2.1 Kirby-Bauer Disc Diffusion

In this experiment, separate Mueller-Hinton agar plates were streaked with each of the 28 different clinical *E. coli* strains and two control strains as described in Chapter 3. The experiments were done in triplicate for each of the 30 strains to test the reproducibility of the data that was measured and recorded in millimetres. **Figure 4.1** below is an example of the typical streaked agar plates containing the tested agents respectively i.e., *B. vulgaris* Ø (test plate) and the antibiotics (control plate). The zones of inhibition are visible and measurements were attained thereof.

The data obtained for the tests performed with the 30 *E. coli* strains is summarised in **Figure 4.3** using Box-and-Whisker plots and is shown all together (**Figure 4.3A**) and without the tincture (**Figure 4.3B**) to show the data on a more representative scale. In descriptive statistics, a box plot is a pictorial tool for graphically portraying sets of numerical data through their quartiles. Box plots too contain lines spreading from the box diagrams which indicate their

variability outside the quartiles (both upper and lower), hence the box and whisker terminology (Persico, 2019).

Before discussing the data obtained it is important to determine if the test results are reliable and this is achieved using the controls included **(Table 4.1)**. The first controls are the negative control included to test and rule out the influence of any of the testing components such as the media, distilled water and blank discs (Lipsitch *et al*., 2011).

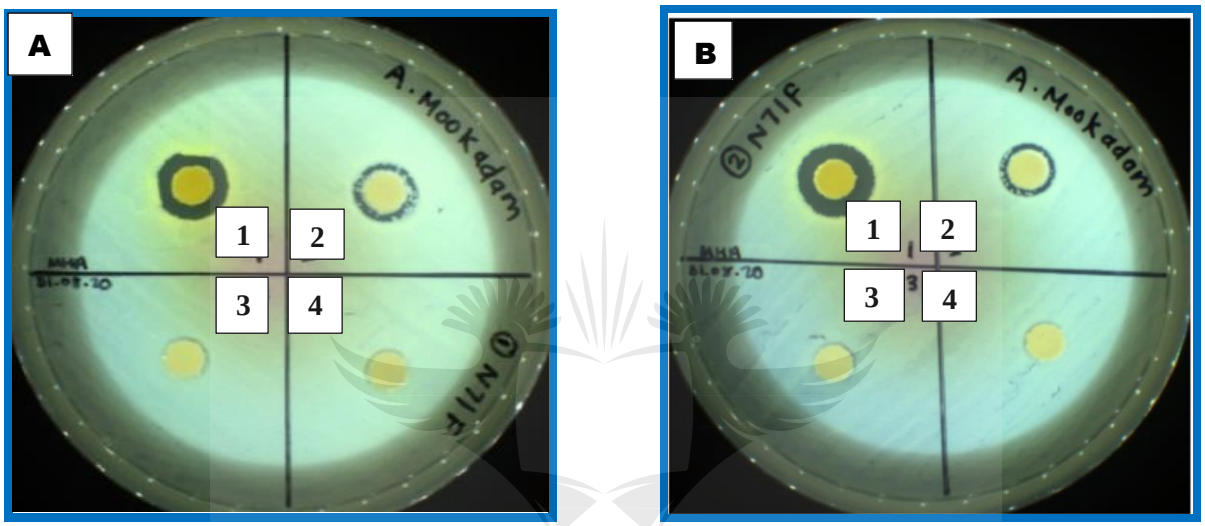


Figure 4.1 Streaked agar plates containing: A1) *Berberis vulgaris* mother tincture (test plate), A2) Ethanol 62%, A3) Distilled water, A4) Blank, B1) Imipenem 10 µg, B2) Cefepime 30g, B3) Distilled water and B4) Blank.

4.2.1.1 Negative controls

A negative control is a part of the trial that is run in parallel to the primary experiment with the same procedures apart from the treatment that is changed to something that is projected to have no result as seen in Figure 4.2.

Table 4.1 Summary of the readings obtained with the Kirby-Bauer Disk Diffusion Susceptibility Tests performed with the *E. coli* strains. The readings are given in millimetres (mm).

Compound	Min	Max	Mean	Median
Negative controls				
Empty disc (blank)	6.0	6.0	6.0	6.0
Water control (Distilled water)	6.0	6.0	6.0	6.0
Positive controls				
Cefepime (30 µg)	9.6	37.2	27	35.4
Imipenem (10 µg)	23.9	35.2	31.9	33.8
Solvent control				
62% (v/v) Ethanol	5.0	12.6	9.1	9.2
Test compound				
B. vulgaris mother tincture	7.7	13.0	11.2	10.6

The negative controls used in this experimental study was a blank disc and distilled water for each of the 30 strains of *E. coli*. These controls were unable to inhibit the growth of either the two reference strains or any of the other 30 strains of *E. coli* tested here confirming that the bacterial inhibition will be attributed to what is added to the disc during the test.

4.2.1.2 Positive Controls

The positive control is the control not exposed to the investigational treatment; however, it is visible to some other treatment that is identified to yield the probable effect (Figure 4.2). In this case the antibiotics Imipenem and Cefepime.

The largest zones of inhibition produced, were visible from Cefepime 30µg at 37.2 mm proving its significant bactericidal activity (**Table 4.1**). The larger the diameters measured, the greater the growth inhibition of the bacterial strains. Cefepime is a mediator that acts by inhibiting the synthesis of the bacterial cell wall and acts on both Gram-positive and Gram-negative microbes (Shah *et al.*, 2016). Thereby producing these prominent outputs.

The other antibiotic positive control Imipenem 10µg displayed an average of 30 mm zone of inhibition. This is indicative of the study by Pap-Wallace *et al*. (2011) which showed that

carbapenems disrupt the microbes' cell wall development and structure, thereby increasing the level of antimicrobial activity. These values exhibit success in the degree of inhibition by both the antibiotics used in this experiment and shows that the correct results can be obtained with the test method if the tincture tested inhibits growth.

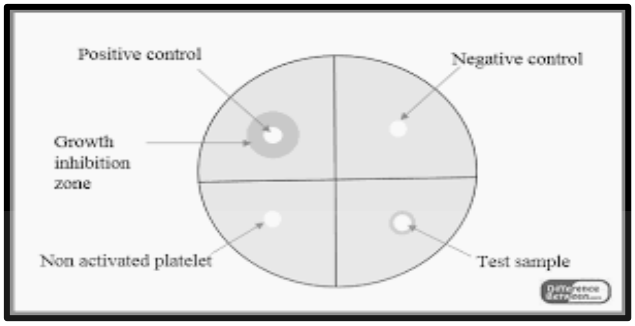


Figure 4.2 Template of the Positive and Negative controls of an antimicrobial Disc Diffusion experiment

4.2.1.3 Solvent Controls

The solvent control used in this experimental study was 62% (v/v) ethanol to test if the inhibition observed with the *B. vulgaris Ø* cannot be attributed to the solvent used to prepare the tincture, i.e., ethanol. The 62% ethanol used in this trial for the test plates did show antimicrobial effect against all 30 bacterial strains producing a maximum of a 12.6 mm zone of inhibition.

The most viable explanation for the antimicrobial action of the alcohol is the denaturation of proteins, which is reinforced by absolute ethyl alcohol being a dehydrating agent (Rutala *et al*., 2019). Protein denaturation is once more consistent with observations that ethanol destroys the dehydrogenases of most of the strains of *E. coli*. *E. coli* and *S. typhosa* were killed within 5-10 seconds by concentrations of ethanol from 40% to 100% (Sharma *et al*., 2017). The blank disc and the distilled water presented with no bacterial inhibition as anticipated.

4.2.1.4 Data Reproducibility

Experiments were conducted in triplicate for all of the thirty bacterial strains to test for repeatability and reliability of the results. As illustrated in **Table 4.2** a very high percentage (90%) of the *E. coli* strains have been inhibited on all three levels of repeatability, which demonstrates that the *B. vulgaris Ø* does produce impeccable reproducibility and prominent antibacterial properties. Ethanol on the other hand has worked at a percentage of 43% of the bacterial strains tested. This proves as a good inhibitory solvent experimentally. However, it does reveal that it only participates minimally in the testing process against *E. coli* strains. When it is associated to *Berberis vulgaris* it does present positively as it is present when the tincture is produced through extraction. The definite antibacterial and therapeutic assets become transparent with *B. vulgaris Ø* on its own in the tincture form and not in association to any complementary solvents.

Table 4.2 Summary of the number of repeats that worked with the Kirby-Bauer Disc Diffusion Susceptibility Tests performed with the *E. coli* strains. The results are shown as percentage (number of samples) of the 30 *E. coli* strains tested.

	***B. vulgaris* mother tincture (Ø)**	**62% (v/v) Ethanol**
No repeats worked	0% (all samples worked)	0% (all samples worked)
One repeat worked	97% (29 samples)	90% (27 samples)
Two repeats worked	97% (29 samples)	70% (21 samples)
Three repeats worked	90% (27 samples)	43.3% (17 samples)
Total	**90% (27 samples)**	**43.3% (17 samples)**

In the diagrams **Figure 4.3 (A and B)**, the blank and distilled water controls both illustrated a norm of a 6mm zone of inhibition during the length of the entire experiment which concludes as expected no inhibition of bacterial growth. The *B. vulgaris Ø* and the solvent 62% ethanol are present in percentages while the two antibiotics are in mg/ml. Cefepime 30 µg displays the

highest level of inhibition and the greatest variability among the 30 *E. coli* strains and therefore the highest antibacterial activity.

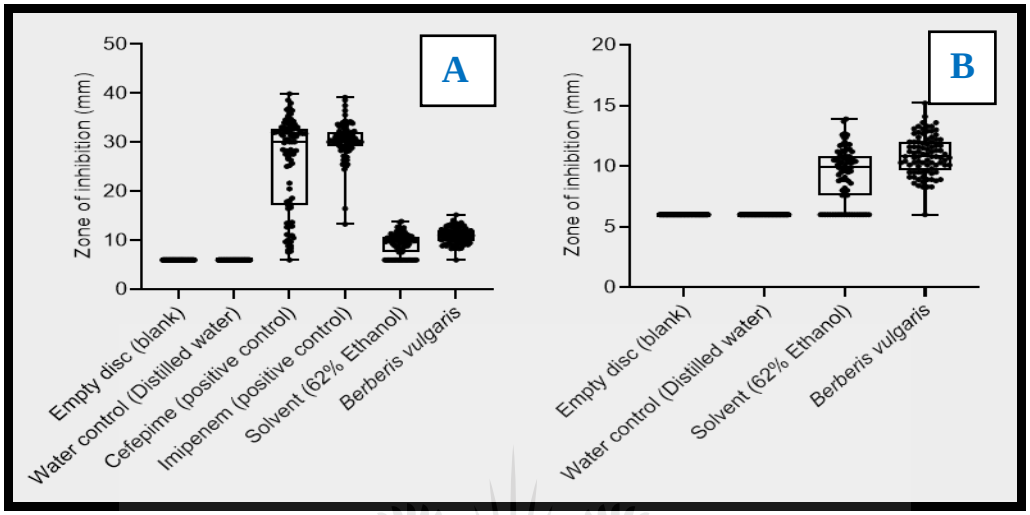


Figure 4.3 Box and Whisker plots display the diameters obtained for the controls, solvent and tincture when tested against the *E. coli* strains.

Following this is the Imipenem 10µg with excellent bactericidal inhibition, however a decreased variability with among the strains as seen in the outliers depicted in **Figure 4.3 A.** Imipenem is active against an extensive range of pathogens, creating its significance in the treatment of serious polymicrobial and mixed aerobic/anaerobic infections, including primary empirical treatment (Rodloff, 2006).

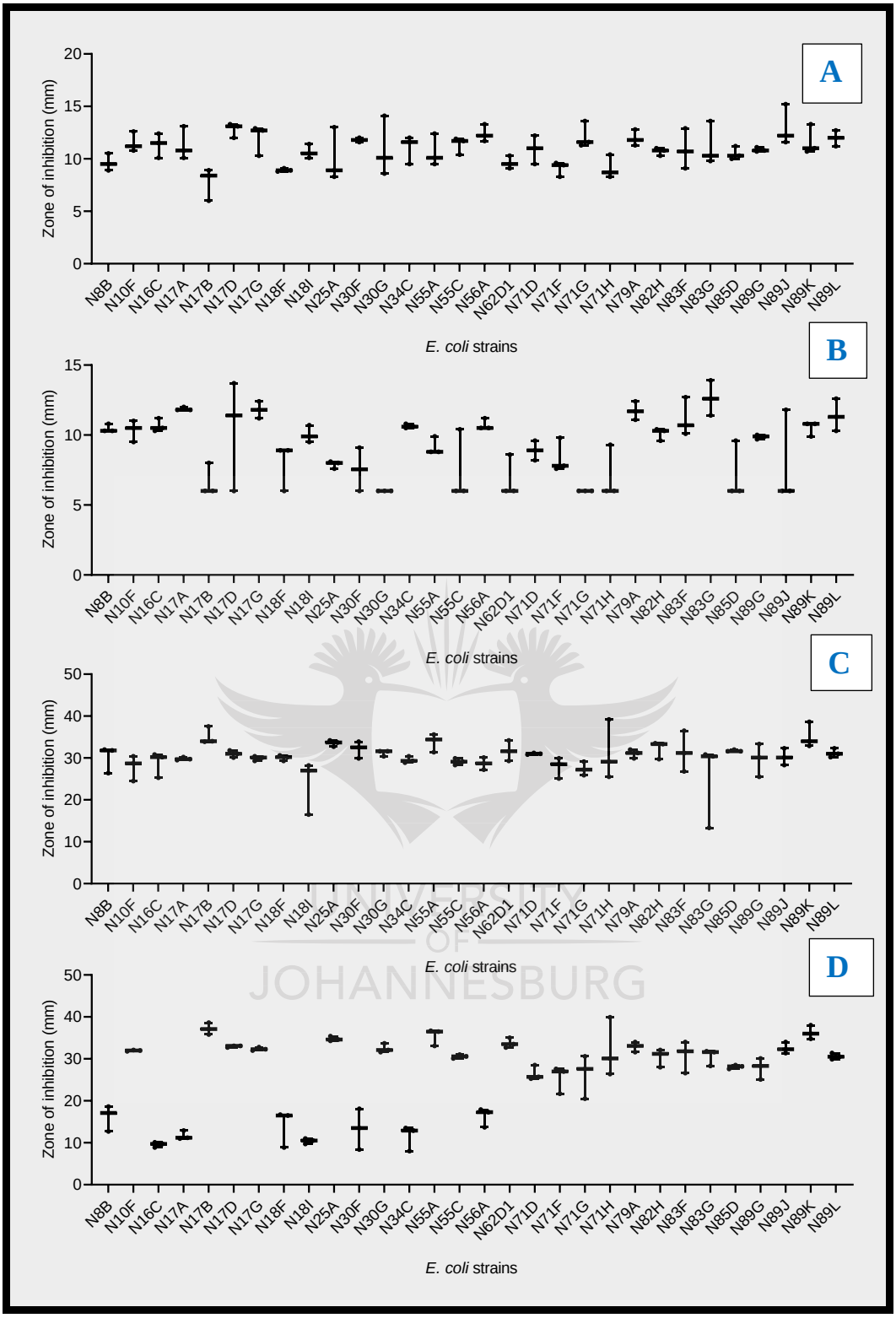


Figure 4.4 Data obtained for each *E. coli* strain exposed to *B. vulgaris Ø* (A), solvent (B), Imipenem (C) and Cefepime (D).

In Graph A present in **Figure 4.4**, *B. vulgaris Ø* produced zones of inhibition ranging between 8-12.5 mm diameters with a few outliers present. This in turn displays an increased consistency between the values and a decreased variability, unveiling its undeniable antimicrobial properties between a very wide range of 30 *E. coli* strains. Dashti *et al*. (2014) demonstrates that the aquatic and ethanolic extracts of *B. vulgaris Ø* with a concentration from 35 to 40 µg/ml disclosed a noteworthy antibacterial effect as minimum inhibitory concentration (MIC) against both gram-positive and negative bacteria. The strains of *Pseudomonas aeruginosa* (MIC=16 microg/ml), *Proteus vulgaris* (MIC=30 microg/ml) and *E. coli* (MIC=32 microg/ml) showed the highest growth inhibition. Berberine, a form of isoquinoline alkaloid with a lengthy history of medicinal properties and applications, is the chief active component of *B. vulgaris Ø* (Belwal *et al*., 2020). Recent studies have proved that berberine has many pharmacological activities such as antioxidant, antimicrobial, antidiarrheal and anti-tumour activity (Tabeshpour, 2017).

The solvent (62% ethanol) has also produced results between the 6-12 mm range, however there were many outliers contributing to the negative skewing in the plotting. This concludes that the ethanol demonstrates a good level of inhibition against the bacterial strains as depicted in **Table 4.2**, however there is an increased degree of variability as it contributes a small percentage to the makeup and composition of *B. vulgaris Ø* as explained in the HAB4A method (Appendix B).

The distribution in both the positive control antibiotics is positively skewed wherein the box plot shows the medians closer to the lower quartile. This means that the data constitute a higher frequency of the high valued scores. Cefepime 30 µg and Imipenem 10 µg in **Figure 4.4** (Graph C and D) respectively, illustrated the largest zones of inhibition and therefore the highest level of antimicrobial activity. Although Imipenem is quite consistent in its ranges, Cefepime shows the most variability among all the constituents tested in this experiment and therefore a potential alternative to the carbapenems for the treatment of infections caused by ESBL-producing bacteria and in paediatric care (Jan *et al*., 2018).

4.2.1.5 Berberis vulgaris mother tincture (Ø)

The antimicrobial effects of *B. vulgaris Ø* were assessed by the degree to which the compounds inhibited the bacterial growth of *E. coli*. The discs utilised in this experiment had 6 mm

diameters, therefore measurements which were displayed as 6 mm in **Table 4.1** validates no inhibition of bacterial growth.

B. vulgaris Ø produced an average between the strains as high as 12.8 mm (inhibition) indicative of the antimicrobial and therapeutic effects against *E. coli*. This positivity is believed to originate primarily from the active compound berberine present in the *B. vulgaris* mother tincture (Madiseh *et al*., 2017).

As visually depicted in **Figure 4.5,** there is no definite correlation between the *Berberis vulgaris* mother tincture (%) and the two antibiotics (mg/ml), which was proved by the statistical analysis. Cefepime 30 µg exhibits the most prominent antimicrobial activity against all the 30 strains of *E. coli* as indicated in the graph with its bacterial resistant properties (resistant and intermediate resistant) in **Figure 4.5 A**. Imipenem 10 µg also possesses a reputable degree with its resistant properties yet indicated susceptibility similar to the tincture. However, *Berberis vulgaris* on its own in tincture form promises excellent antimicrobial and therapeutic properties among many others with the slightest expectation of side effects (Rad *et al*., 2017).

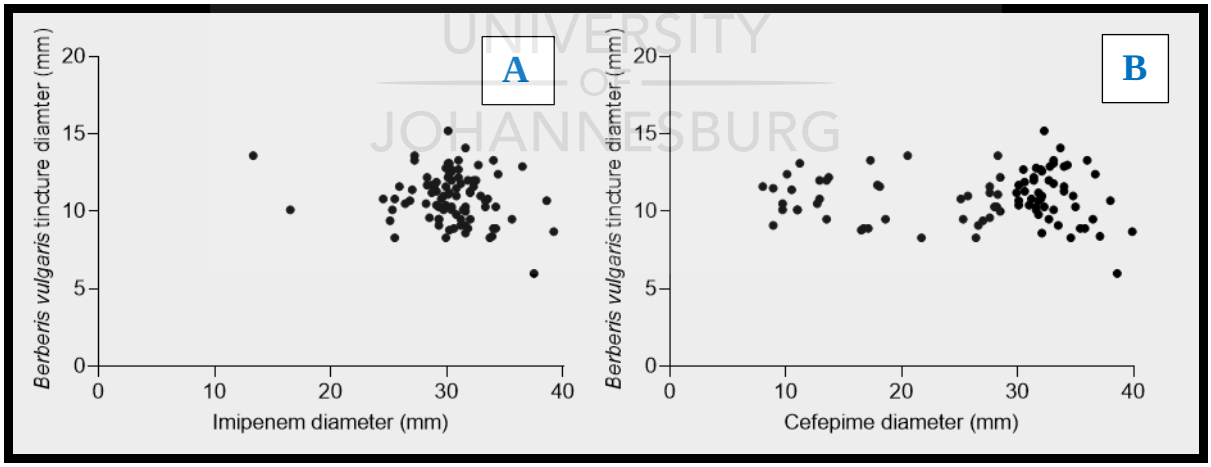


Figure 4.5 The relationship between the tincture *B. vulgaris* and Imipenem (A) and Cefepime (B) respectively.

The prominent antibacterial effects of *Berberis vulgaris* L. were proven in a few recent studies. An experiment by Ekhtelat *et al*. (2020) tested the antibacterial activity of *Berberis vulgaris* L. and *Foeniculum vulgare* extracts independently and in combination with sodium diacetate and nisin against *E. coli* O157:H7 strain. Nisin displayed the maximum antibacterial activity against *E. coli* O157:H7 followed by the extracts of *B. vulgaris* L. and *Foeniculum vulgare*, and lastly sodium diacetate. The facts and figures were consistent with the outcomes of the disc diffusion trial.

An *in-vitro* bactericidal activity of *Berberis aristata* extract against clinical isolates of carbapenem-resistant *E. coli* was tested by Thakur *et al.* (2016) and found to contain a number of phytoconstituents which act in a synergistic manner to provide significant bactericidal potential against carbapenem-resistant *E. coli*.

The antimicrobial activity of the bark on the root of *Berberis lycium* and its primary element berberine, which is the same active constituent of our experiment in *B. vulgaris Ø*, was tested against a varied set of microbial strains by means of the agar disc diffusion test and further analysed using microdilution technique. By examining the zone of inhibition, it disclosed that the methanolic extract of *B. lycium* was highly effective against *E. coli* (zone of inhibition = 41 ± 1 mm). Amongst the strains tested, *E. coli* was found to be most vulnerable and from the fungal category *Candida albicans* was the most susceptible for berberine as well as the crude methanolic extract of the plant (Malik *et al*., 2017). According to Malik *et al.* (2017), in this study the potentiation of this berberine by resistance breaking molecules in the crude extract could be a probable explanation for its strong effectiveness against the *E. coli* bacterial strains.

4.2.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) assay is a method used to illustrate how a specific antibiotic in its lowest concentration is required to inhibit bacterial growth (Kowalska-Krochmal *et al*., 2021). It is usually accomplished on free floating bacterial cells. These microdilution procedures are utilised in susceptibility testing and also to determine the MICs of numerous antimicrobial agents. Bacteria are tested for their capability to yield growth on the microtitration plate wells comprising of sequential dilutions of the antimicrobials.

The concept is that the bacteria is grown in wells containing the media with or without a test compound or antibiotic. The test compound is typically serial diluted by taking a set volume from one well, mixing it with the contents of the second well before removing the same volume and going to the next well. This is repeated a set number of times, with the portion removed from the last well discarded. This microdilution technique was used to confirm the output attained from the Kirby-Bauer disc diffusion susceptibility test and to thereafter determine the MIC where there was bacterial growth inhibition. The compounds were verified by serial dilution method in 96 well plates as explained previously in Chapter 3. The tests were conducted on 30 *E. coli* bacterial strains.

Figure 4.6 below illustrates the *E. coli* strains present in the distinctive 96 well plate layout used to test the compounds using the microdilution process. All of the wells contained the *E. coli* strains apart from the media control. The wells that produced a purple colour subsequently to the addition of the Iodonitrotetrazolium chloride (INT) dye presented with bacterial growth therefore, signifying no inhibition of *E. coli* bacterial growth by the compounds. The wells that presented with no colour transformation after the addition of the test compound and INT dye indicates no bacterial growth, hence the inhibition of the bacteria by the compounds as depicted in **Table 4.3** below.

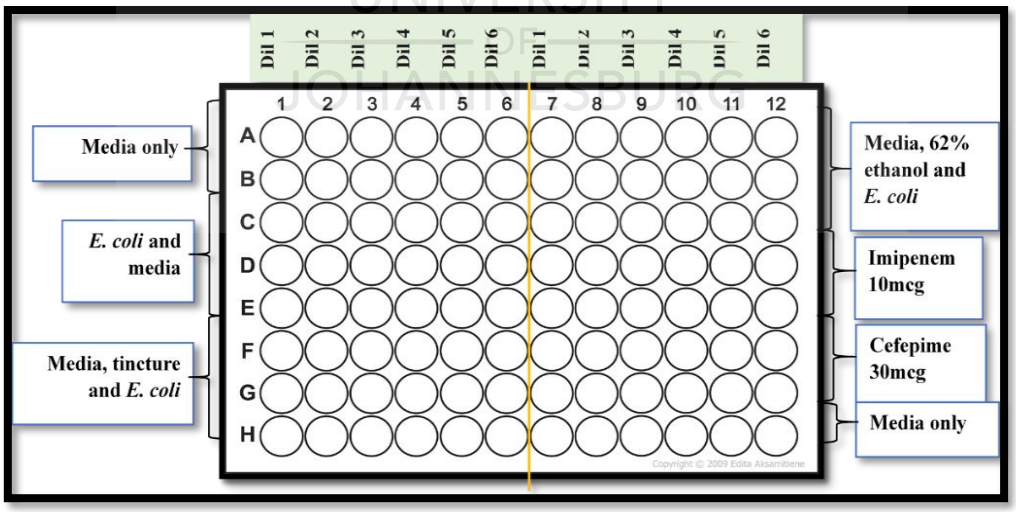


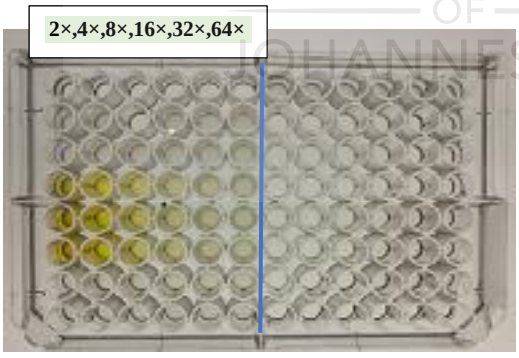
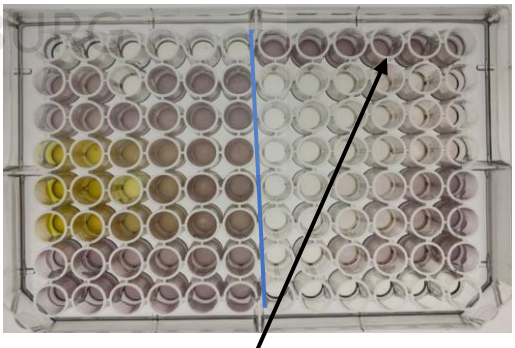
Figure 4.6 Template of the 96 well plate and its contents used to determine the MIC of *B. vulgaris* mother tincture on the *E. coli* strains.

Once the strains of *E. coli* interact with the compound it can now to move away from the test compound creating the distinct halo effect. This halo effect intensifies as we move across each of the wells consecutively, thus as the concentration of the tincture decreases, as will the halo. All 30 strains of *E. coli* have the ability to participate in bacterial swarming, where the bacterium moves away due to its motility by the flagella over a surface to save itself (Swiecicki *et al*., 2013).

4.2.2.1 Media Control

The minimal inhibitory concentration (MIC) test is accomplished using Mueller- Hinton Agar (MHA), which is the best medium for routine susceptibility testing owing to the fact that it possesses a decent reproducibility and is low in sulphonamide, trimethoprim and tetracycline inhibitors that could influence the test results. This media control indicates if there was any contamination of the media while adding to the plates that could influence the results. No growth was observed for any of the media controls on all the plates run (**Table 4.4)**.

Table 4.3 Example of the 96 wells plates before and after the INT dye illustrating bacterial growth.

Sample	Before INT dye	After INT dye
E. coli Control 2		

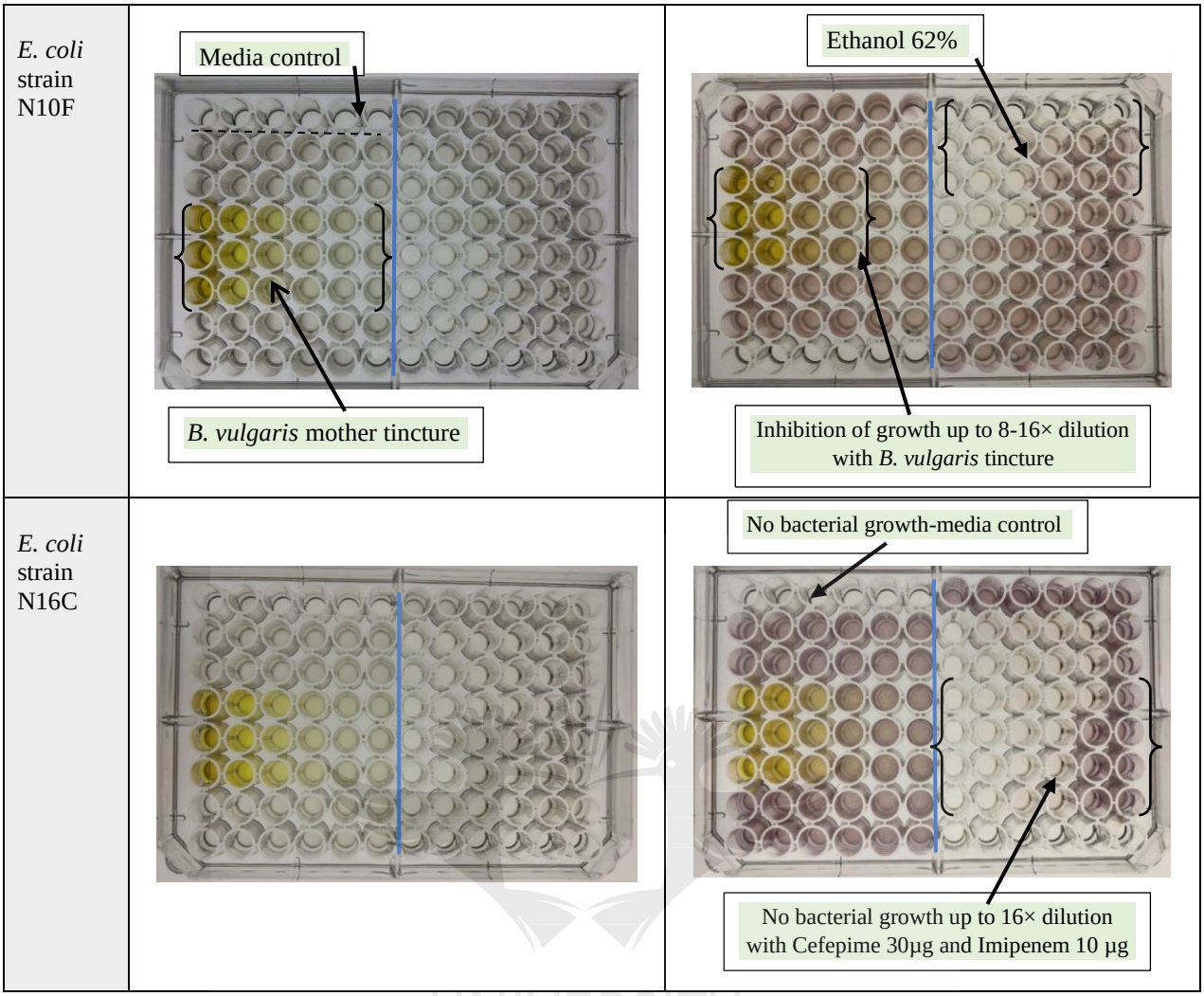


Table 4.4 Summary of the values attained in percentage (%) from Statistical analysis of the MIC test.

Compound	Min	Max	Mean	Median
Media control	0%	0%	0%	0%
Bacteria controls	100%	100%	100%	100%
Positive controls				
Cefepime (30 µg)	0%	3%	0.7%	0.01%
Imipenem (10 µg)	0%	1%	0.2%	0%
Solvent control				
62% (v/v) Ethanol	0.02%	15%	3.3%	0.02%
Test compound				
B. vulgaris mother tincture	0.02%	4.5%	1.05%	0.02%

4.2.2.2 Bacteria controls

The bacteria controls are grown in only media to ensure that the bacteria can grow under the test circumstances. There was growth on the plates for the two reference strains and all of the other 28 strains of *E. coli* tested here (**Table 4.4)**, confirming that the bacterial isolates can grow and that growth inhibition will be credited to what is added to the consecutive wells as the dilution process continues.

4.2.2.3 Positive Controls

Offering to the expected outcome is the fact that both these antibiotics employ bactericidal activity by interfering with bacterial cell wall synthesis and thereby preventing peptidoglycan cross-linking. Bactericidal properties result through inhibition of cell development and division, thereby resulting in the loss of cell wall integrity and eventually causing cell wall lysis.

Both Imipenem and Cefepime presented a maximum bacterial growth inhibition at 1% and 3% antibiotic dilution respectively, therefore possessing the ability to inhibit the total growth of the bacterial strains tested by a rate of 97%.

In **Figure 4.7** there appears to be very little correlation between the tincture and the two antibiotics data both statistically and visually, however, all 3 embrace prominent bacterial resistant qualities. This was compared to determine if similar survival strategies could have been used in the presence of the test compound. It was discovered that the results from both the Kirby Bauer disc diffusion and the microdilution (MIC) processes showed a decent degree of inhibition by the *Berberis vulgaris* mother tincture and therefore positive antibacterial effects against 90% of the 30 strains of *E. coli* tested.

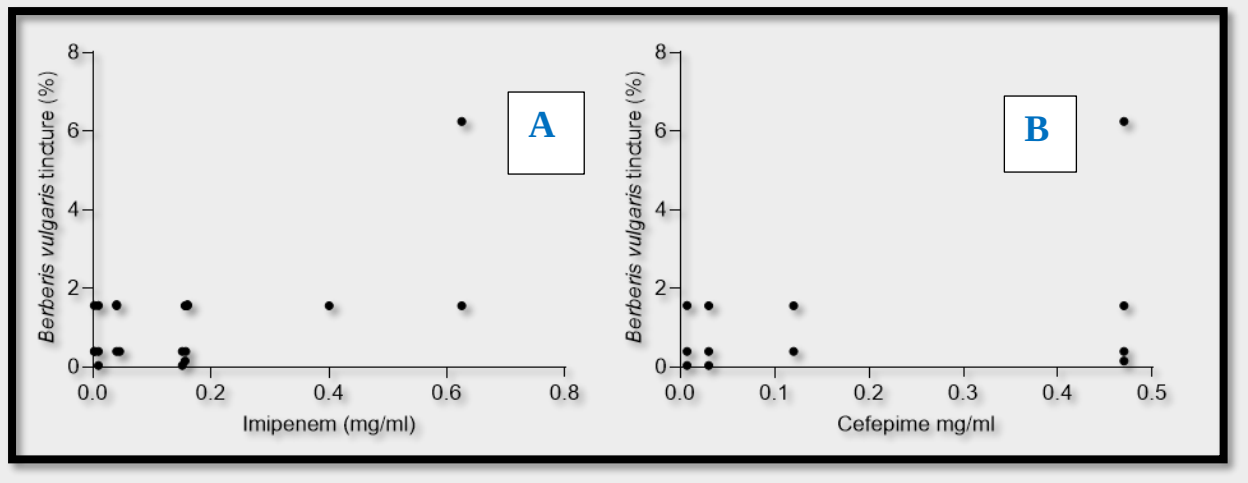


Figure 4.7 Relationship between tincture (%), Imipenem (A) and Cefepime (B)(mg/ml).

4.2.2.4 Solvent Controls

The 62% ethanol used in this test did confirm the antimicrobial effect against all 30 bacterial strains producing a maximum concentration of inhibition at 15% (v/v) ethanol and a minimum growth of 0.02% (v/v) ethanol. This positivity emanated throughout the repeats in the entire testing procedure. Ethanol 62%–95% can denature the proteins of microbes and therefore enhance their ability to inactivate bacteria and viruses (Jing *et al*., 2020) and this should be taken into consideration when describing the potential effect of the mother tincture prepared in this solvent.

4.2.2.5 B. vulgaris mother tincture

The *B. vulgaris Ø* only showed inhibition until the second and fourth dilution levels, as no noticeable change was seen from yellow to purple in most of thirty-two bacterial strains thereafter. The purple colour change occurred from the 16× dilution onward indicating that bacterial growth has started again.

There is limited literature to support optimal antimicrobial effect seen with the *B. vulgaris Ø* thus far in the previous studies conducted. It can thereby be acceptable to conclude that with a

greater concentration and dosage of the tested compound, the potential to inhibit the strains of *E. coli* could be greater.

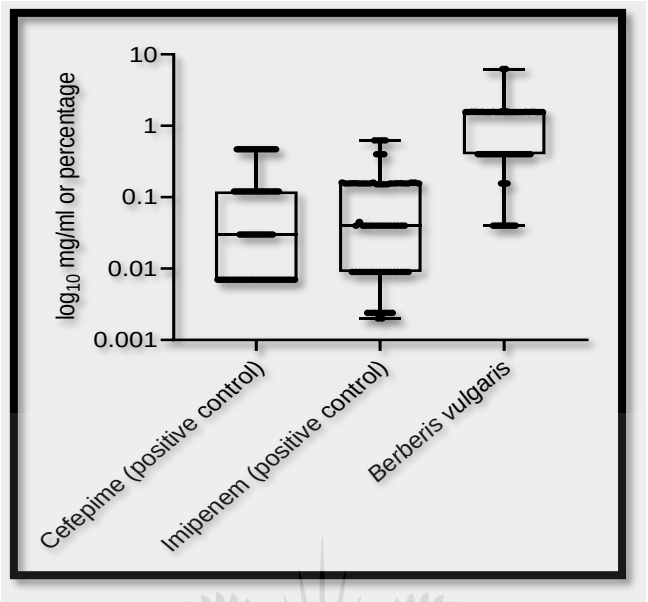


Figure 4.8 Box and Whisker plot for the *E. coli* strains exposed to the two antibiotics (Imipenem 10µg and Cefepime 30 µg) and the *B. vulgaris* tincture. The *B. vulgaris* tincture is presented in percentages while the antibiotics are indicated in mg/ml.

Evidently **Figure 4.8** demonstrated a somewhat symmetric distribution for both the antibiotics, however a slightly negatively skewed plotting for the tincture is visible. Cefepime displayed the highest resistance and least susceptible to the 30 *E. coli* strains, while imipenem was intermediately resistant and *Berberis* showed intermediate resistance and susceptibility to the strains**.**

The figure (**Figure 4.9**) below was drawn using GraphPad Prism version 9. The tincture *B. vulgaris* (Graph A) indicates an admirable consistency with the antimicrobial action between the 30 bacterial strains and a minimal number of outliers from the values attained in the MIC. Graph B and C respectively represent the antibiotics and were calculated using a log10 scale. Both Imipenem 10 µg and Cefepime 30µg boasted in **Figure 4.9** an increased level of variability amongst the strain's indicative of its profound antimicrobial properties.

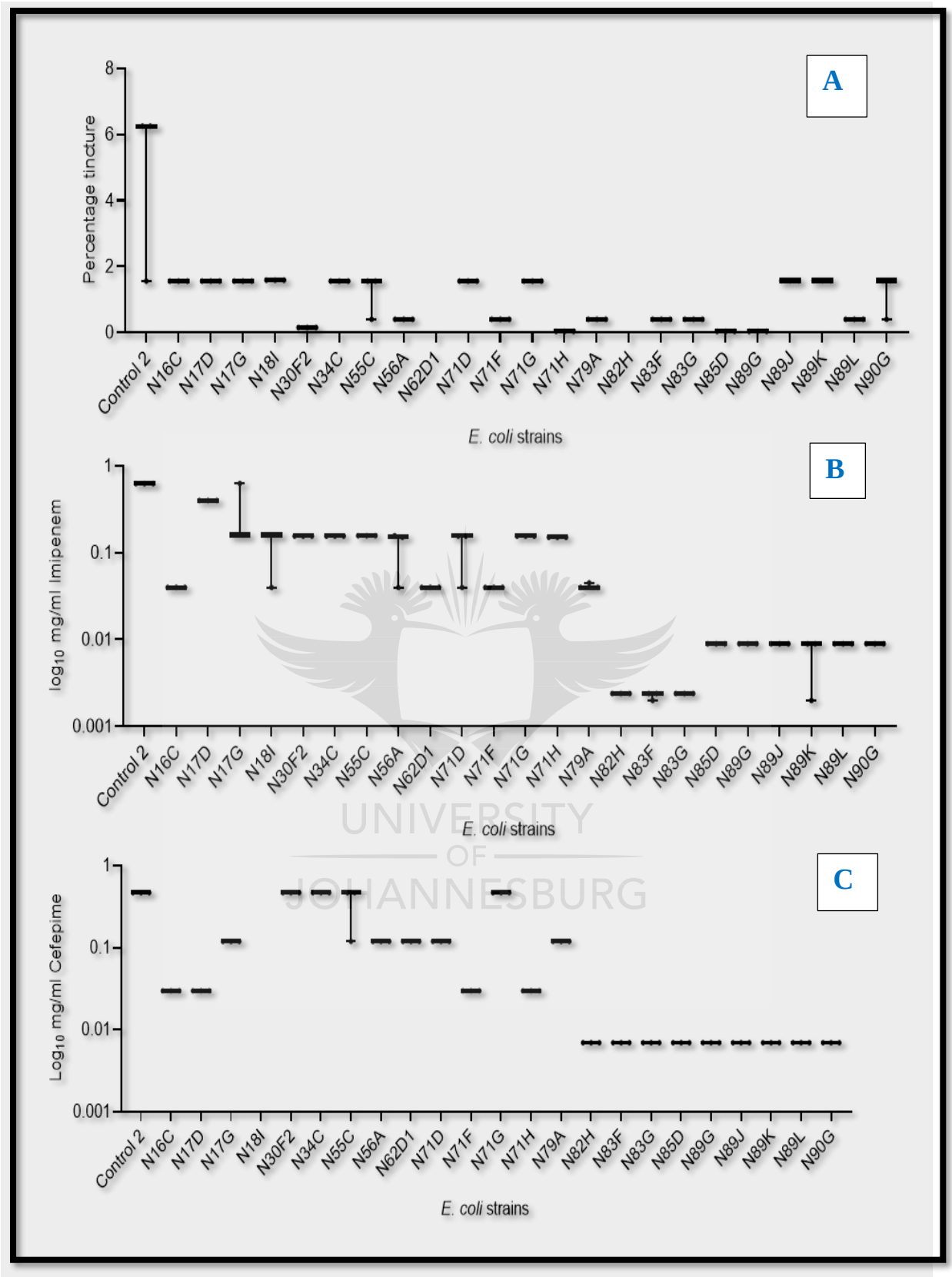


Figure 4.9 The Minimum Inhibitory Concentrations obtained for the tincture (A), Imipenem (B) and Cefepime (C) against the *E. coli* strains tested

Boberek *et al*. (2010) in a recent study demonstrated the effects of berberine on *E. coli* by the use of the microdilution process to determine the MIC and Fluorescence Microscopy testing. Outcomes disclosed that berberine had formed filaments, signifying an antimicrobial mechanism that comprises cell division inhibition. Berberine was found to target and thereby inhibit bacterial division proteins which stop bacteria from multiplying, consequently contributing to the antimicrobial effects.

The minimal inhibition and low antimicrobial activity achieved from the *B. vulgaris Ø* and at times the antibiotics during the experimental process according to Swiecicki *et al*. (2013), may point to the fact that at decreased concentrations of the antimicrobial agent the microbes have the ability to protect themselves by creating a barrier to absorb the effect of the compound and then start to disperse, surrendering cells at the surface and thereby preserve the nutrients and be perceived as dead.

The less it was diluted and the higher the concentration of the compounds, the higher the degree of inhibition and antimicrobial activity against the bacteria by both the tincture and antibiotics. There have been additional reports of similar results obtained in previous trials reported in Dashti *et al*. (2004), Freile *et al*. (2006) and Malik *et al*. (2016).

The descriptive statistics were performed to obtain the summaries of the data. Parametric tests included the Kolmogorov-Smirnova test of normality and one sample T-test. Non-parametric test included the Friedman test and Wilcoxon Signed Ranks Test. The statistical tests showed there was no normal distribution of the data and that there were no significant differences observed for the test compound in comparison to the antibiotics.

Overall, the results obtained displayed a confirmation that the effects of the *B. vulgaris* mother tincture is bacteriostatic, however this depends on the concentration of the compound added. *Berberis vulgaris* displayed significant inhibition against 90% of all thirty-two bacterial strains of *E. coli* which was visible due to the production of measurable zones of inhibition produced around the discs each time (Kirby-Bauer) and the minimal colour change (MIC). It does require further investigation as a candidate for its antibiotic properties. These results however, suggest that this tincture promises a possible alternative to antibiotics in the future, as evidenced by its antimicrobial properties exhibited in this study.

4.3 Limitations of the Study

Mentioned below are some possible limitations of this study:

- Minimum control over variables (systematic errors during the collection and analysis of data) that might bias the outcome and results of this experiment which makes it problematic for another researcher to replicate the study for further research purposes.
- The cultures of the bacterial suspensions that were produced 24 hours before the experimentation or longer, may not compare to the expected bacterial counts and values as at times prepared cultures were >24 hours.
- The inability to calculate active constituents of the test compound.
- Time constraints - there was a limited duration for certain aspects of the experimental process that had to be completed within that time frame and were not carried out due to other aspects of the experimental process that had to be redone/revised.
- Human error during the experiment, unknowingly or not recognized during the trial, that may affect the outcome.

CHAPTER 5

5. CONCLUSION AND RECOMENDATIONS

5.1 CONCLUSION

This quantitative *in-vitro* study aimed to assess the antibacterial effects of *B. vulgaris Ø* on *E. coli in-vitro*, by means of the Kirby-Bauer Disc Diffusion and the Microdilution methods to confirm the minimum inhibitory concentrations (MICs).

The results showed that *Berberis vulgaris* Ø displayed significant inhibition against all thirty-two bacterial strains of *E. coli* which was visible due to the production of measurable zones of inhibition produced around the discs each time. This was established by the Kirby-Bauer disc diffusion method which was carried out in triplicate ensuring optimal results during the experimentation processes. The microdilution method (96 well plate technique) has also displayed positive feedback on most of the strains, as visible in previous studies contributing and verifying the prominent antimicrobial and medicinal effects of *B. vulgaris Ø.*

Further investigation in this study, with similar methodology and an increase in the concentration/dosage of the tincture could obtain a more successful outcome by not just inhibiting but killing the bacteria instead. It was hypothesised that the homeopathic mother tincture of *Berberis vulgaris* would demonstrate antimicrobial effects against the *E. coli* strains by inhibiting the growth of the organisms on both solid and liquid culture media which was reinforced and confirmed by this study.

A positive conclusion can be drawn from this study, and it is therefore reasonable to state that *B. vulgaris* Ø did yield a significant degree of inhibition of bacterial growth against a decent percentage of the 30 *E. coli* strains utilised in this study.

This success has provided a stepping stone into further antimicrobial research involving *Berberis vulgaris* and other homoeopathic mother tinctures, exploring their bacteriostatic and bactericidal effects. This, in turn supports the medicinal, therapeutic and antimicrobial effects of utilizing homeopathic mother tinctures, which potentially offers a safe and effective alternative treatment option to antibiotics.

5.2 RECOMMENDATIONS

Below are some recommendations to ensure a higher degree of success in further research based on this study:

- Select higher concentrations/ increased dosages of the homeopathic tincture used against the bacteria.
- *B. vulgaris* Ø could be utilised in conjunction with another tincture or extract to increase the bactericidal effects.
- Additional tinctures could be used/added to test for antimicrobial effects with the same testing methods from this study.
- A similar study could be carried out to recognize why the growth inhibition differs within different strains of the same bacterial species.
- Different testing procedures could be used to test for the antimicrobial effects of *Berberis vulgaris* and other tinctures.
- The tincture could be tested on other types of bacteria with different concentration levels to expose the most susceptible bacterial strains.
- Test the active ingredients of the tincture/ medicine before initiating the experimental process.

REFERENCES

Akhavan, B.J., Khanna, N., Vijhani, P. (2020). Amoxillin. *Stat Pearls Internet Publishing*. no. NBK482250.

Ameer, MA., Wasey, A. and Salen, P. (2020). Escherichia Coli (E Coli 0157 H7). *Stat Pearls Internet Publishing LLC*. vol.1, pp.622.

Arayne, M.S., Sultana N., & Bahadur, S. (2007). The berberis story: Berberis vulgaris in therapeutics. *Pakistan journal of pharmaceutical sciences*. vol.20, pp. 83-92.

Aryal, S. (2020). McFarland Standards- Principle, Preparation, Uses, Limitations. *Basic Microbiology.* pp.1-3.

Azwanida, N. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Medicinal and Aromatic Plants*. vol.3, no.4, pp. 196.

Bachir, D., and Abouni, B. (2015). *Escherichia coli and Staphylococcus aureus most common source of infection.* Available at: http://www.microbiology5.org/microbiology5/book/637-648. [Last accessed 20 January 2020].

Baquero, F. and Levin, B.R. (2020). Proximate and ultimate causes of the bactericidal action of antibiotics. *Nature Reviews Microbiology*. vol. 19, pp.123–132.

Baron, S. (1996). Medical Microbiology. *Galveston (TX): University of Texas Medical Branch at Galveston.* vol.4, pp.20-35.

Barr, W., and Smith, A. (2014). Acute Diarrhoea in Adults. *Lawrence Family Medicine Residency, Lawrence, Massachusetts. AM Family Physician.* vol.89, no.3, pp. 180-189.

Baughman, RP. (2009). The use of carbapenems in the treatment of serious infections. *Journal of intensive care medicine.* vol.24, no.4, pp.230-241.

Belwal, T., Bisht, A., Devkota H.P., Ullah, H., Khan, H., Pandey, A., Bhatt, I.D. and Echeverría, J. (2020). Phytopharmacology and Clinical Updates of *Berberis* Species Against Diabetes and Other Metabolic Diseases. *Frontiers in Pharmacology*. vol. 11, pp. 41.

Bloch, R. & Lewis, B. (2008). The Law of Similars. In A van Rooyen, (Ed). *Homeopathy for the home second edition.* South Africa: Bloch, R. & Lewis, B. pp.24.

Brazier, Y. (2017). *E. coli* Infections: Symptoms, causes and Treatment. *Medical News Today*.UK. pp. 2-3.

Boberek, J., Stach, J. and Good, L. (2010). Genetic evidence for inhibition of bacterial division protein FtsZ by berberine. *PLoS ONE*. vol.5, no.10, pp.13745.

Bundy, LM and Noor, A. (2019). Neonatal Meningitis. *Stat Pearls Internet Publishing LLC*.no. NK532264.

Carolli, K., Morse, S., Mietzner, T., Miler, S: Jawitz, Melnick & Adelberg's Medical Microbiology 27th Edition. Available at: http:// www.accessmedicine.com. [Last accessed February 2020].

Cefepime Alpha pharm Consumer Medicine Information (CMI) (2017). *Cefepime (cephalosporin antibiotic) Indications*. Available at: http://www. myvmc.com/drugs/cefepime/. [Last accessed 01 April 2020].

Centres for Disease Control and Prevention(CDC) (2020). How Antibiotic/Antimicrobial Resistance Happens. *National Centre for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Healthcare.* Available at:

https://www.cdc.gov/drugresistance/about/how-resistance-happens.html. [Last accessed May 2021].

Cepada-Molero, M., Berger, CN., Alistair D. S. Walsham, Ellis, SJ., Wemyss-Holden,S,. Schüller, S., Frankel, G and Fernández, LA. (2017). Attaching and effacing (A/E) lesion formation by enteropathogenic *E. coli* on human intestinal mucosa is dependent on non-LEE effectors. *Plos pathogens*. vol.13, no.10, pp.1-3.

Chatuphonprasert, W., Lao-Ong, T. and Jarukamjorn, K. (2013). Improvement of superoxide dismutase and catalase in streptozotocin- nicotinamide-induced type 2-diabetes in mice by berberine and glibenclamide. *Pharmaceutical Biology*. vol.52, no.4.

Chavez, B.J. and Tadi, P. (2021). Gentamicin. *Stat pearls Publishing LLC.* Bookshelf ID: NBK557550.

Cirino, E. (2019). *What you need to know about Herbal Tinctures.* Available at: http://www.healthline.com/health/what-is-a-tincture/. [Last accessed October 2020].

Clements, A., Young, J.C., Constantinou, N., and Gad Frankel. (2012). Infection strategies of enteric pathogenic *Escherichia coli. Gut.* vol. 3, no.2, pp. 71–87.

Cockerill, F.K., Wilker, M., Alder, J., Eliopoulus, G., Hardy, D., Hecht, D., Hindler, J. (2012). Performance Standard for Antimicrobial Disc Susceptibility Test, Approved Standard-Eleventh Edition. *Clinical and Laboratory Standard Institute*. vol.29, no.1, pp.8-47.

Das, E. (2019). Understanding the Principles of Homeopathy on A Research Perspective. *Journal of Complementary Medicine and Alternative Healthcare*. vol.9, no.2.

Dashti, Z., Nabi, S., Abdolreza, and N, Mohammedi. (2014). Study on antibacterial and antioxidant activity of Berberis vulgaris aqueous extracts from Iran. *International Journal of Pharma Sciences and Research*. vol.5, no. 10, pp.705.

Davis, N. (2018). *The human microbiome: Why our microbes could be key to our health.* Available at: http://www.theguardian.com/new2018/. [Last accessed 29 September 2020].

Ekhtelat, M., Shima, A.B., Forough, N. and Abdolghani, A. (2020). The Antibacterial Activity of Barberry Root and Fennel Seed Extracts Individually and in Combination with Nisin and Sodium Diacetate Against Escherichia coli O157: H7. *Jundishapur Journal of Natural Pharmaceutical Products.* vol 15, no.1.

Eloff, J.N. (1998). A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria. *Department of Pharmacology, University of Pretoria, Pretoria.* vol.1, no.64, pp.711-713.

Etsy Inc.com (n.d.). *Berberis vulgaris*. Available at: https://www.etsy.com/market/berberis_vulgaris . [Last accessed June 2021].

Fair, RJ., and Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st Century. *Perspectives in medicinal chemistry*. vol. 6, pp. 25-64.

Fatima, R., Aziz, M. (2019). Enterohemorrhagic Escherichia Coli (EHEC). *Centres for Disease Control and Prevention, National Centre for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED)*.

Flores-Mireles A. L., Walker J. N., Caparon M. and Hultgren S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat. Rev. Microbiology*. vol.13, pp. 269–284.

Freile-Pelegran, Y. and Morales, J.L. (2006). Antibacterial activity in marine algae from the coast of Yucatan, Mexico. *Botanica Marina*. vol.47, no.2, pp.140-146.

Friedman, N.D., Temkin, E., and Carmeli, Y. (2016). The negative impact of antibiotic resistance. *Clinical Microbiology and Infection*. vol.22, no.5, pp.416-422.

Ganesan, T., Ravi, D.B., Vasavan, J., Khurana, A., Nayak, D. and Peranakan, K. (2015). Homoeopathic preparation of Berberis vulgaris as an inhibitor of Calcium oxalate crystallization: In vitro evidence. *Indian Journal of Research in Homeopathy*. vol.9, no. 3, pp. 152-157.

Gaynes, R. (2017). The Discovery of Penicillin—New Insights After More Than 75 Years of Clinical Use. *Emerging Infectious Diseases*. vol. 23, no.5, pp. 849–853.

Fu, Z., Iglay, K., Qiu, Y. and Engel, S. (2014). Risk Characterization for Urinary Tract Infections in Subjects with Newly Diagnosed Type 2 Diabetes. *Journal of Diabetes and its Complications.* vol.28, no.6.

Gbenga, J. (n.d.) *The Truth About Infections In both Men and Women with Special Emphasis on Sexually Transmitted Infection (STI's) and Urinary Tract Infection (UTI's)*. Available at: http://www.everfitness.com.ng/urinary.html. [Last accessed May 2021].

Gerba, C.P. (2014). Environmentally Transmitted Pathogens. *Environmental Microbiology.* no.10, pp. 509–550.

German Homoeopathic Pharmacopoeia. (1993). General Regulation for manufacturing homoeopathic drugs. In: B.H. Association, (Ed). *German Homoeopathic Pharmacopoeia.* London: Dutscher Apotheker Verlag Stuttgart. pp. 27-32.

Gomes, T.A.T., Elias W.P.,Isabel, C.A., Scaletsky ., Guth, B.C., Rodrigues, J.F., Piazza, R.F., Ferreira, LCS., and Martinez, MB. (2016). Diarrheagenic Escherichia coli. *Brazilian journal of microbiology. Us National library of medicine.*

Gossman, W., Wasey, A. and Salen, P. (2019). *Escherichia coli* (E coli 0157 H7). *Stat Pearls Publishing, Treasure Island (FL).* pp. 299-39622.

Gupta, P.D. and Birdie, T.J. (2017). Development of botanicals to combat antibiotic resistance. *Journal of Ayurveda and Integrative Medicine.* vol.8, no.4, pp. 266-275.

Hain, T.C., Cherchi,M. and Yacovino, D.A. (2018). Bilateral Vestibular Weakness. *Frontier Neurology*. vol.9, pp.344.

Hamao,N.,Ito,I.,Konishi,S., Tanabe, N.,Shirata, M.,Oi, I.,Tsukino,M., Matsumoto, H.,Yasuto mo,Y., Kadowaki, S., and Hirai ,T. (2020). Comparison of ceftriaxone plus macrolide and ampicillin/sulbactam plus macrolide in treatment for patients with community-acquired pneumonia without risk factors for aspiration: an open-label, quasi-randomized, controlled trial. *BMC Pulmonary Medicine* .vol. 20, no.160.

Handa, S.S., Khanuja, S.P.S., Longo, G. and Rakesh, D.D. (2008). Extraction technologies for medicinal and aromatic plants. *Earth, Environmental and Marine Science and Technologies.* Trieste (Italy). vol.3, no.8, pp. 260.

Huang, J., Duan, Q. and Zhang, W. (2018). Significance of Enterotoxigenic *Escherichia coli* (ETEC) Heat-Labile Toxin (LT) Enzymatic Subunit Epitopes in LT Enterotoxicity and Immunogenicity. *Applied and environmental microbiology. American society for microbiology.* vol.8, no.49, pp.18.

Hudzicki, J. (2013). *Kirby-Bauer Disc Diffusion Susceptibility Test Protocol*. Available at: http://www.microbelibrary.org/component/resoursce/laboratory-test/3189/kirby-bauer-disc-diffusion-susceptibility-test-protocol. [Last accessed February 2020].

Humphries, R. M. and Linscott, A. J. (2015). Laboratory diagnosis of bacterial gastroenteritis. Clinical Microbiology. vol.28, no.1, pp. 3-3.

Jan, S., Ragunanthan, B., DiBrito, S.R., Alabi, O. and Gutierrez, M. (2018). Cefepime Efficacy and Safety in Children: A Systematic Review and Meta-analysis. *Frontiers in Paediatrics.* vol.10, no.3389.

Jing, J.L.J., Pei Yi, T., Bose, R.J.C., McCarthy, J.R., Tharmalingam,N. and Madheswaran,T. (2020). Hand Sanitizers: A Review on Formulation Aspects, Adverse Effects, and Regulations. *International Journal of Environmental Research and Public Health.* vol.17, no.9, pp.3326.

Kapoor, G., Saigal, S., and Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology, Clinical Pharmacology*. vol. 33, no.3, pp.300-305.

Kapwata, T., Mathee, A., Jacobus le Roux, W. and Wright, C.Y,. (2018). Diarrhoeal Disease in Relation to Possible Household Risk Factors in South African Villages. *International Journal of Environ Res Public Health*. vol.15, no.8, pp. 1665.

Kaushik, D., Mohan, M., Borade, D.M. and Swami, O.C. (2014). Ampicillin: Rise Fall and Resurgence. *Journal of Clinical and Diagnostic Research*. vol. 8, no.5, pp.1-3.

Kim, K.S. (2003). Meningitis-associated Escherichia coli. *Basic Medical Key- Johns Hopkins University School of Medicine, Baltimore.* pp.10.

Kowalska-Krochmal, B and Dudek-Wicher, R. (2021). The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. *Department of Pharmaceutical Microbiology and Parasitology, Faculty of Pharmacy, Medical University of Silesian Piasts.* vol.211, no.50, pp.556.

Kushner, B.S., Allen, P.D. and Crane, B.T. (2017). Frequency and Demographics of Gentamicin Use. *Otology Neurotology*. vol.37, no.2, pp.190-195.

Ku, C.L., Boggess, KA. and Cohen-Wolkowiez, M. (2016). Bacterial Meningitis in the Infant. *Clinical Perinatology*. vol.42, no.1, pp.29-45.

Leimbach, A., Hacker, J. and Dobrindt, U. (2013). *E. coli* as an all-rounder: the thin line between commensalism and pathogenicity. *Current Topics in Microbiology and Immunology.* pp. 3-32.

Li, X.Z., Plésiat, P. and Nikaido, H., (2015). The Challenge of Efflux-Mediated Antibiotic Resistance in Gram-Negative Bacteria. *American society of Microbiology*. vol.28, no.2, pp. 337.

Lipsitch, M., Tchetgen, E. and Cohen, T. (2011). Negative Controls: A Tool for Detecting Confounding and Bias in Observational Studies. *Epidemiology. Author manuscript.* vol.21, no.3, pp. 383-388.

Lupindu, AM. (2016). Isolation and Characterization of Escherichia coli from Animals, Humans, and Environment. *E. coli- Recent Advance on Physiology, pathogenesis and Biotechnological applications.* vol.10, no.5772, pp.1-3.

Mac, K., Chavada, R., Paull, S.,Howlin, K. & Wong, J. (2015). Cefepime induced acute interstitial nephritis – a case report. *Bio Med Central Nephrology*. vol. 16, no.15.

Madiseh, M.R., Lorigoini, Z., Zamani, H., & Mahmoud, R. (2017). Traditional and specific uses of Berberis vulgaris. *Iran J Basic Med Sci.* pp.569–587.

Malik, T.A., Kamili, A.N., Chishti, M.Z., Ahad, S., Tantry, M.A., Hussain, P.R., Johri, R.K. (2017). Breaking the resistance of *Escherichia coli*: Antimicrobial activity of Berberis lycium Royle. *Microbial pathogenesis*. pp. 12-20.

Manosalva, L., Mutis, A., Urzúa, A., Fajardo, V. and Quiroz, A. (2016). Antibacterial Activity of Alkaloid Fractions from Berberis microphylla G. Forst and Study of Synergism with Ampicillin and Cephalothin. *Molecules (Basel, Switzerland*). vol.2, no.1, pp.76.

McLellan , L.K. and Hunstad, D.A. (2016). Urinary Tract Infection: Pathogenesis and Outlook. *Trends Molecular Medicine.* vol. 22, no.11, pp. 946–957.

Mohammadzadeh N., Mehri S. and Hosseinzadeh H. (2017). Berberis vulgaris and its constituent berberine as antidotes and protective agents against natural or chemical toxicities. *Iranian Journal of Basic Medical Sciences*. Vol.20, no.5, pp. 538-551.

Mokomane, M., Kasvosve, I., de Melo, E., Pernica, J.M., and Goldfarb, D.M. (2017). The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management. *Us National library of medicine. Therapeutic advances in infectious disease.*

Najar, M.S, Saldanha, C.L and Banday, K.A. (2009). Approach to urinary tract infections. *Indian Journal of Nephrology*. vol.19, no.4, pp. 129-139.

Neag, M.A., Mocan,A., Echeverría, J., Pop, R.M., Bocsan, C.I., Crişan G., and Buzoianu, A.D .(2018). Berberine: Botanical Occurrence, Traditional Uses, Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders. *Frontiers in Pharmacology*. vol.10, no.33, pp.89.

Newman, T. (2018). *How do Penicillins work?* Available at: https://www.medicalnewstoday.com/articles/216798. [Last accessed January 2020].

Nicolau, D. P,. (2008). Carbapenems: a potent class of antibiotics. *Expert opinion on pharmacotherapy.* vol.9, no.1, pp.23-37.

Odonkor, S.T. and Joseph, K. A (2013). Escherichia Coli as an Indicator of Bacteriological Quality of Water: An Overview. *Microbiology Research*. pp. 33.

Ogbru, O. (2019). *Cipro, Cipro XR Antibiotic.* Available at: https://www.medicinenet.com/ciprofloxacin/article.htm. [Last accessed February 2021].

Papp-Wallace, K.M., Endimiani, A.,Taracila, M.A and Bonomo, R.A. (2011). Carbapenems: Past, Present, and Future. *American Society for Microbiology-Antimicrobial Agents and Chemotherapy.* vol.55, no.11, pp. 4943-4960.

Pasqua, M., Michelacci, V., Di Martino, M.L., Tozzoli, R., Grossi, M., Colonna, B.,Morabito, S., and Prosseda, G. (2017). The Intriguing Evolutionary Journey of Enteroinvasive *E. coli* (EIEC) toward Pathogenicity. *Frontier Microbiology.* vol. 8, pp.2390.

Peechakara, B., Basit., H., Gupta, M., (2020). Ampicillin Article. *Stat Pearls Internet Publishing*. no. 17481.

Persico, A. (2019). *Box and Whisker Plots explained in 5 easy Steps*. Available at: https://www.mashupmath.com/blog/box-and-whisker-plots-explained. [Last accessed 3 March 2021].

Piddock , L.J.V. (2006). Clinically Relevant Chromosomally Encoded Multidrug Resistance Efflux Pumps in Bacteria. *Clinical Microbiology Revised*. vol. 19, no.2, pp. 382-402.

Rad, S., Rameshrad, M. and Hosseinzadeh, H. (2017). Toxicology effects of *Berberis vulgaris* (barberry) and its active constituent, berberine: a review. *Iranian Journal of Basic Medical Science.* vol. 20, no. 5, pp. 516–529.

Rasheed, MU., Thajuddin N., Ahamed, P., Teklemariam, Z and Jamil, K. (2014). Antimicrobial drug resistance in strains of *E. coli* isolated from food sources. *Rev Inst Med Trop Sao Paulo.* vol. 56, no.4, pp. 341–346.

Ratini, M. (2021). *What is Homeopathy?* Available at: http://www.webmd.com/balance /what-is-homeopathy. [Last accessed 3 March 2021].

Reis, A.C.C., Santos, S.D., deSouza, S.C., Saldanha, M.G., Pitanga, T.N., and Oliviera, R.R. (2016). Ciprofloxacin Resistance pattern among bacteria isolated from patients with

community acquired urinary tract infection. Revised Inst. Medicine Trop. Sao Paulo. vol. 58, pp. 53.

Reygaert, WC, (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology.* vol. 4, no.3, pp. 482-501.

Rodloff, AC., Goldstein, E.J.C. and Torres, A. (2006). Two Decades of Imipenem Therapy. *The Journal of Antimicrobial Chemotherapy*. vol.58, no.5, pp.916-929.

Rutala, W.A., and Weber, D.J. (2019). Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. *Centres for Disease Control and Protection*. pp.11-39.

Sabina, M. Vranic and Aida, Uzunovic (2016). Antimicrobial resistance of *E. coli* strains from urine at output populations. *A single laboratory experiences*. pp.121-124.

Saka, H.K., Dabo, N.T., Muhammad, B., Garcia-Soto, S., Ugarte-Reiz, M., and Alvarez, J. (2019). Diarrheagenic *Escherichia coli* Pathotypes from Children Younger Than 5 Years in Kano State, Nigeria. *Frontiers in Public Health*. no. 7, pp. 348.

Saxena, P. (2021). How Do Antibiotics Work: Mode & Mechanism of Action of Antibiotics? Available at: http:// http://healthlove.in/antibiotics/how-do-antibiotics-work-mode-mechanism-of-action-of-antibiotics/. [Last accessed May 2021].

Schepper, L.D. (). Hahnemann Revisited: A Textbook of Classical Homeopathy for the Professional. *Revised Edition- from Homeopathy Today*. vol.20, no.4.

Schmiemann, G., Kniehl, E., Gebhardt, K., Matejczyk, M.M., and Hummers-Pradier, E,. (2010). The Diagnosis of Urinary Tract Infection, A Systematic Review. *Dtsch Arztebl International.* vol.107, no.21, pp. 361–367.

Shah, M., Shah, H. and Maslekar, A. (2016). Cefepime induced neurotoxity in a patient with coronary artery bypass. *Indian Journal of pharmacology*. vol.48, no.5, pp 599-600.

Shahid, T., Memon, M., Malik, R.A., Ikram, N., Malik, W., & Ali, A. (2017). A study of Antimicrobial Activity of *Berberis vulgaris* (Zirishk) Aqueous Plant Extract using Pathogenic Isolates from patients of Islamabad and Rawalpindi. vol.3, no.5, pp. 2454-1362.

Sharma, D., Patel, R.P., Zaidi, S.T.R., Sarker, M.M.R., Lean, Q.Y., and Ming, L.C. (2017). Interplay of the Quality of Ciprofloxacin and Antibiotic Resistance in Developing Countries. *Frontiers in Pharmacology*. vol. 8, pp.546.

Sharma, C., Aneja, K.R. and Kasera, R. (2011). Screening of Berberis Aristata DC. For Antimicrobial Potential against the Pathogens causing Ear Infection. *International Journal of Pharmacology*. vol.7, pp.536-541.

Shurtleff, David. (2018). *What Is Homeopathy?* Available at: https://www.nccih.nih.gov/health/homeopathy. [Last accessed 15 January 2020].

Simon, A.K., Hollander, G. and McMichael, A. (2015). Evolution of the immune system in humans from infancy to old age. *Proc. Biological Sciences*. vol.282, no.1821.

Singh, M., Srivastava, S., Rawat, A.K. (2007). Antimicrobial activities of Indian Berberis species. *Fitoterapia.* vol .78, no.8, pp.574-6.

Storme, O., Saucedo, J.T., Garcia-Mora, A., Dahesa-Davila, M and Naber, K.G. (2019). Risk factors and predisposing conditions for urinary tract infection. *Therapeutic advances in Urology*. vol.11, PMC6502981.

Swiecicki, J., Sliusarenko, O. and Weibel, D. (2013). From swimming to swarming: *Escherichia coli* cell motility in two-dimensions. *Integrative Biology*. vol. 5, no.12, pp.1490.

Tabeshpour, J., Imenshahidi, M. and Hosseinzadeh, H. (2017). A review of the effects of *Berberis vulgaris* and its major component, berberine, in metabolic syndrome. *Iranian Journal of Basic Medical Science.* vol. 20. no.5, pp. 557–568.

Tan, C.W. and Chlebicki, M.P. (2016). Urinary tract infections in adults. *Singapore Medical Journal.* vol.57, no.9, pp. 485–490.

Tang, B.H., Wu, Y.E., Kou, C., Qi, Y.J., Qi, H., Xu, H.Y., Leroux, S, Huang, X, Zhou, Zheng, Y., Jacqz-Aigrain, E, Shen, A.D., and Zhao, W. (2019). Population Pharmacokinetics and Dosing Optimization of Amoxicillin in Neonates and Young Infants. *American society for Microbiology, Antimicrobial agents and chemotherapy*. vol.63, no.2, pp. 18.

Tang, Y., Kim, H., Singh, A.K., Aroonnual, A., Bae, E., Rajwa, B. (2014). Light Scattering Sensor for Direct Identification of Colonies of Escherichia coli Serogroups O26, O45, O103, O111, O121, O145 and O157. *State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology*, China. vol 9, no.8 pp. 105272.

Tangcharoensathien, V., Chanvatik, S., Sommanustweechai, A. (2018). Complex determinants of inappropriate use of antibiotics. *Bulletin of the World Health Organisation*. vol.17, pp.141-144.

Terlizzi, M.E., Gribaudo, G., and Maffei, M.E. (2017). Uropathogenic *Escherichia coli* (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-antibiotic Antimicrobial Strategies. *Frontier Microbiology.* vol. 8, pp. 1566.

Thakur, P., Chawla, R., Narula, A., Goel, R., Arora, R and Sharma, R.K. (2016). *In vitro* bactericidal activity of *Berberis aristata* extract against clinical isolates of carbapenem-resistant *Escherichia coli. Journal of Complementary and Integrative Medicine.* vol.13, no.3, pp.229-237.

Tounier, A., Klein, S.D., Würtenberger, S., Wolf, U. and Baumgartner, S. (2019). Physicochemical Investigations of Homeopathic Preparations: A Systematic Review and

Bibliometric Analysis—Part 2. *Journal of Alternative and Complimentary Medicine*. vol.25, no.9, pp. 890-901.

Utt, E., and Wells, C. (2016). The Global response to the threat of Antimicrobial Resistance and the important role of vaccines. *Pharmaceuticals Policy and Law*. vol. 18, pp. 179-197.

Varley, A.J., Sule, J., Absalom, A.R. (2009). Principles of antibiotic therapy. *Continuing Education in Anaesthesia Critical Care & Pain*. vol. 9, no. 6, pp.184–188.

Verma, S., Qian, Z., Adhya, S. (2019). Architecture of the *Escherichia coli* nucleoid. *Plos Genetic Journals.* vol.10, no.08456.

Ventola, A.L. (2015). The antibiotic resistance crisis. *Pharmacy and therapeutics*. vol.40, no.4, pp.277-283.

Vermeulen, F. (2005). Prisma. *The Netherlands. Emryss*. pp. 240.

World Health Organisation (WHO). (2015). *Antibiotic resistance*. Available at: http:// www.who.int/mediacentre/factssheets/antibioticresistance/en/. [Last accessed 03 April 2020].

APPENDIX A

WATER AND HEALTH RESEARCH CENTRE
FACULTY OF HEALTH SCIENCES

Water and Health Research Centre
PO Box 17011
Doornfontein
2028

18 September 2019

To: Chairperson of the FHS HDC

Re: Approval for Azraa Mookadam to conduct her research in the WHRC

Dear Prof S Nalla

This is to confirm that Azraa Mookadam been granted laboratory space and benches to conduct her research in the Water and Health Research Centre. She will be working on her Master's project entitled "*The antibacterial effect of Berberis vulgaris mother tincture on Escherichia coli in vitro*" and will be supervised in the laboratory by trained personnel.

Please feel free to contact me directly for further information or clarification.

Yours sincerely,

Prof TG Barnard
Director: Water and Health Research Centre
Faculty of Health Sciences
Tel 072 579 5748
Fax 011 559 6342
Email tgbarnard@uj.ac.za

APPENDIX B

Berberis vulgaris mother tincture preparation according to HAB 4A method (German Homeopathic Pharmacopoeia, 2013).

- Using this method tinctures are normally prepared by maceration or percolation.
- One part of the dried root and 10 parts of ethanol of appropriate concentration.
- **By maceration**: comminute the dried root and mix with ethanol of appropriate concentration i.e., 70% in this case. Allow to stand in a closed container for an appropriate time. Separate the residue from the ethanol and if necessary, press out. Lastly combine the 2 liquids obtained.
- **By percolation**: If necessary, comminute the herbal drug/root. Mix thoroughly with a portion of ethanol of appropriate concentration and allow standing. Transfer to a percolator and permit the percolate to flow slowly at room temperature. Make sure the drug to be extracted is always covered with the remaining alcohol. The residue may be pressed out and the expressed liquid is combined with the percolate.
- If adjustment to a concentration is necessary, calculate the amount in kilograms(kg) of ethanol of the appropriate concentration required to obtain the concentration specified or used for production using the following expression:

M × (NX- NO) ÷ NO

- M= mass of percolate/macerate.
- NX= percentage of dry residue/assay content of percolate/macerate.
- NO= percentage of dry residue/assay content of percolate/macerate as required in the individual monograph.
- Mix macerate/percolate with calculated amount of ethanol with appropriate concentration. Allow to stand for not less than 5 days and thereafter filter.
- Final alcohol percentage present in the tincture is 60-62%.

APPENDIX C

E. coli strains stored at the WHRC were used for this study. This included two reference strains (ATCC11775 and ATCC 8739) and 30 strains named below:

1. N25A	2. N71F
3. N56A	4. N71D
5. N85D	6. N83G
7. N82H	8. N89L
9. N17B	10. N55C
11. N17A	12. N71H
13. N10F	14. N89J
15. N61D	16. N89K
17. N17D	18. N60F
19. N55A	20. N30F2
21. N18F	22. N16C
23. N62D1	24. N17G
25. N83F	26. N34C
27. N30G	28. *E. coli* control 1
29. N89G	30. *E. coli* control 2

APPENDIX D

FACULTY OF HEALTH SCIENCES

RESEARCH ETHICS COMMITTEE

NHREC Registration: REC 241112-035

ETHICAL CLEARANCE RENEWAL LETTER

(RECX 3.0)

Student/Researcher Name	Mookadam, A	Student Number	201144922
Supervisor Name	Dr J Pellow	Co-Supervisor Name	Prof TG Barnard
Department	Complementary Medicine		
Research Title	THE ANTIBACTERIAL EFFECT OF BERBERIS VULGARIS MOTHER TINCTURE ON ESCHERICHIA COLI IN-VITRO		
Previous Clearance Date	19 November 2019	Clearance Number	**REC-181-2019**
Date	1 February 2021		

Approval of the research with details given above is renewed and is valid until 1 February 2022.

1. Conditions:
None.

2. Renewal:
It is required that this ethical clearance is renewed annually, within two weeks of the date indicated above. Renewal must be done using the Ethical Clearance Renewal Form (REC 10.0), to be completed

and submitted to the Faculty Administration office. *See Section 12 of the REC Standard Operating Procedures*.

3. Amendments:

Any envisaged amendments to the research proposal that has been granted ethical clearance must be submitted to the REC using the Research Proposal Amendment Application Form (REC 8.0) prior to the research being amended. Amendments to research may only be carried out once a new ethical clearance letter is issued. *See Section 13 of the REC Standard Operating Procedures*.

4. Adverse Events, Deviations or Non-compliance:

Adverse events, research proposal deviations or non-compliance must be reported within the stipulated time-frames using the Adverse Event Reporting Form (REC 9.0). *See Section 14 of the REC Standard Operating Procedures*.

The REC wishes you all the best for your studies.

Yours sincerely.

Prof. Christopher Stein

Chairperson: REC
Tel: 011 559 6564

Email: cstein@uj.ac.za

APPENDIX E

FACULTY OF HEALTH SCIENCES

HIGHER DEGREES COMMITTEE

C-01-99-2019

25 November 2019

TO WHOM IT MAY CONCERN:

STUDENT: **MOOKADAM, A**
STUDENT NUMBER: 201144922

TITLE OF RESEARCH PROJECT: **The Antibacterial Effect of Berberis Vulgaris Mother Tincture on Escherichia Coli in- Vitro**

DEPARTMENT OR PROGRAMME: **COMPLEMENTARY MEDICINE**

SUPERVISOR: **Dr J Pellow** **CO-SUPERVISOR:** **Prof TG Barnard**

The Faculty Higher Degrees Committee has scrutinised your research proposal and concluded that it complies with the approved research standards of the Faculty of Health Sciences; University of Johannesburg.

The HDC would like to extend their best wishes to you with your postgraduate studies.

Yours sincerely,

Prof S Nalla

Chair: Faculty of Health Sciences HDC

Prof S Nalla

Tel: 011 559 6258

Email: shahedn@uj.ac.za

APPENDIX F

Turnitin Originality Report

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Similarity by Source

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