ELEMENTAL COMPOSITION AND ANTIBACTERIAL EFFICACY OF *MORINGA OLEIFERA* AND *ZINGIBER OFFICINALE* ROOT POWDERS AGAINST *E. COLI*

by

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B.Sc., Punjabi University, Patiala, India, 2018

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
NATURAL RESOURCES AND ENVIRONMENTAL STUDIES

UNIVERSITY OF NORTHERN BRITISH COLUMBIA

March 2022

Abstract

Shortage of clean drinking water is a serious problem faced by the world at present times, with water borne diseases claiming 5 million deaths globally each year. Many methods are available to treat contaminated drinking water; however, cultural, economic, and social factors often impair implementation of these methods, particularly in developing countries. *Moringa* and ginger root powders appear to offer a promising alternative to treat contaminated drinking water. This study examines the nutrient composition of *Moringa* and ginger roots and determine their antibacterial efficacy against *Escherichia coli* (*E. coli*). After growing *Moringa* and ginger plants in a greenhouse for seven months and three years respectively, their roots were harvested, dried, powdered and analysed for their chemical composition using ICP-MS and Elemental analysis. The most abundant metal found in both the root powders was potassium, while calcium, magnesium, sulphur, sodium, and phosphorus were other elements present in high amounts. However, the concentration of these metals in *Moringa* and ginger root powders varied significantly. The abundance of essential elements in the two root powders justifies their use as a point-of-use water treatment method. For both *Moringa* and ginger root powders, 1400 mg/L concentration was determined to be the most effective concentration as it reduced *E. coli* in contaminated water by 88.66% and 62.63% respectively. The combination of the two root powders appeared to have a synergistic effect on *E. coli* as *Moringa* and ginger root powders combined in 1:1 ratio reduced bacterial counts by 94.78% when added to the contaminated water at 2000 mg/L.

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Acknowledgements

First and foremost, I am indebted to my supervisor, Dr. Christopher Opio, for his guidance and supervision throughout the course of this research. I am extremely grateful for every critical feedback that I received from him, as it has immensely helped me to become a better researcher. I am also thankful to him for having patience in me and believing in me to complete this work. Without his moral and academic guidance, this work would not have been possible. I would like to extend my thanks and appreciation to my committee members, Dr. Saphida Migabo and Dr. Natalie Linklater, for their encouraging comments and feedback, and for letting me borrow countless lab equipment from their respective labs all through my research.

I would like to thank Prof. Amarjeet Bhullar for encouraging me to undertake my master's degree at UNBC and for his continual encouragement. I would like to acknowledge funding support from University of Northern British Columbia through Graduate Project Award, which made it possible for me to apply myself completely to my project. I would also like to sincerely thank John Orlowsky and Doug Thompson for their help in the greenhouse; Lon Kerr for graciously providing an *E. coli* strain; and Che Elkin for his assistance in statistical analysis. Data was collected in the Northern Analytical Laboratory Services (NALS) lab at UNBC with the help of Hossein Kazemian, Erwin Rehl and Charles Bradshaw.

I would also like to thank my family and friends for their moral support which helped me immensely during this journey. A special thanks to my sister, for always believing in me.

Chapter 1: Introduction

Quite aptly called the 'Elixir of Life' (Mythrey et al., 2012), water is one of the most important natural resources on this planet (Aulenbach, 1968), without which life would have never existed on the face of the Earth. Its level of purity determines the quality-of-life people lead as it has a direct impact on human health (Abiyu et al., 2018). Lack of clean drinking water, also referred to as potable water, is a huge problem faced by many underdeveloped and developing countries (Varkey, 2018). According to the World Health Organization (WHO), water borne diseases claim 5 million deaths globally each year (WHO, 2019). Most of these deaths are attributed to a wide range of microorganisms including *Escherichia coli*, a common microbe found in contaminated water (Cabral, 2010).

Many methods are available to treat contaminated drinking water; however, cultural, economic, and social factors often impair implementation of these methods, particularly in developing countries (Morgan et al., 2019). Conventional water treatment procedure used in developed countries, which includes coagulation, flocculation, sedimentation, filtration, and disinfection, renders water free of particulate matter such as bacteria, algae, fungi, parasites, organic matter and metals like iron, arsenic (Abiyu et al., 2018). In addition to the above-mentioned procedures, municipal plants in developed countries involve the use of chemical coagulants such as ferrous chloride ($FeCl_2$) and aluminium sulphate [$Al_2(SO_4)_3$], for treating water. These chemical coagulants can adversely affect human health, for instance, aluminium is a recognized neurotoxin that can cause neurodegeneration (Maya et al., 2016) and it has been indicated to be a causative agent of neurological diseases like pre-senile dementia (Abiyu et al., 2018). Therefore, natural coagulants and antimicrobial agents are moving from fringe to mainstream use with majority of people seeking water treatment remedies free of chemical side effects.

Various plants and their products have gained considerable attention for their potential to purify water without exploiting expensive and detrimental chemicals (Dubey et al., 2004). These remedial plants appear to offer a promising alternative to treat contaminated water in developing countries, where most of the rural population depends on contaminated and turbid water from rivers, streams, and dams (Varkey, 2018).

Moringa oleifera is one of the most useful trees in the world that belongs to Moringaceae family (Arora et al., 2013). It is a drought-tolerant tree found in tropical and sub-tropical countries such as India, Mexico, Philippines, Florida and in many parts of Africa (Daba, 2016). It is often described as a 'Miracle Tree' as almost every part of the tree- leaves, flowers, pods, seeds, seed oil, bark etc., has nutritional, medicinal and industrial applications (Daba, 2016).

Leaves, flowers, and immature pods of *Moringa* are consumed by people in countries such as India, Hawaii, Pakistan and many parts of Africa (Daba, 2016). Various parts of the tree are used as circulatory and cardiac stimulants; they contain antileptic, antipyretic, anti-inflammatory, anti-tumour, cholesterol lowering, antidiabetic, antioxidant, antimicrobial properties; and are used for treating various diseases in indigenous medicine system (Anwar et al., 2007).

Various parts of *Moringa* shoot possess antibacterial properties, especially the seeds, which have been extensively studied for their antibacterial and water purifying efficacy. Seed extract of *Moringa* has shown to kill 90-99.99% of bacteria in turbid water (Lea, 2010) and in sedimented sludge (Madsen et al., 1987). However, very little research has been done to study the antimicrobial and water purifying efficacy of *Moringa* roots. Utilizing *Moringa* roots in antimicrobial research would offer additional benefits as roots can be harvested within the first year of planting the trees (Morgan et al., 2019) and without hindering the growth of the plant

(Fuglie, 2001). This would also keep in reserve the nutrient-rich parts of the shoot like leaves and seeds to be used as a food source (Morgan et al., 2019).

The only research done to evaluate the antibacterial efficacy of *Moringa* root powder used three concentrations (250, 450 and 600 mg/L) and found that the maximum root powder concentration (600 mg/L) reduced *E. coli* colonies by 87% in contaminated pond water (Morgan et al., 2019). Likewise, antibacterial efficacy of ginger (*Zingiber officinale*) against *E. coli* and numerous other bacteria such as *Staphylococcus aureus, Salmonella typhi* and *Vibrio cholerae* has been reported (Jagetia et al., 2003; Chrubasik et al., 2005; Gupta and Ravishankar, 2005). Ginger is native to Southeast Asia but is now cultivated in other tropical and subtropical regions such as the West Indies, Africa, Latin America, and Australia (Singletary, 2010). It is commonly used in folk medicine and is known to possess a wide range of pharmacological properties (Afzal et al., 2001).

The objectives of this research were (1) to chemically analyse roots of *Moringa* and ginger plants grown in a greenhouse; (2) to determine the individual effects of *Moringa* and ginger root powders on *E. coli* population when they were added to contaminated water at a concentration higher than 600 mg/L; and (3) to study the combined effects of *Moringa* and ginger root powders on *E. coli* population.

By increasing the concentrations of *Moringa* and ginger root powders (>600 mg/L) in contaminated water, the percentage decrease observed in *E. coli* population was expected to be more than 87% (more than what was achieved by Morgan et al., 2019). It was also anticipated that by mixing the two root powders together in 1:1 ratio, they might have a synergistic effect on *E. coli* population and depict a higher antibacterial efficacy than attained by either of the root powders alone, as bioactive components of plant extracts have ability to modify or block the resistance mechanism of a bacteria when used together (Stefanovic, 2017).

A solution to the problem of inaccessibility of clean drinking water is sought out by many organizations and state governments. The findings of this research might provide a potential solution to the purification of drinking water in underdeveloped countries and rural areas of developing countries where the problem of contaminated water is quite alarming (Varkey, 2018). This research will expand the current knowledge on drinking water purification by identifying the most effective treatment to reduce *E. coli* population to the lowest level possible using *Moringa* and ginger root powders.

Chapter 2: Literature review

2.1 *Moringa oleifera*

2.1.1 Botanical description

Moringa (Moringa oleifera) is native to tropical and sub-tropical regions of South Asia and Africa (Oliveira et al., 1999) and is the most widely cultivated species of the family Moringaceae (Arora et al., 2013). It is commonly identified as drumstick tree, horseradish tree, benzoil tree and cabbage tree in different regions of the world (Koul and Chase, 2015). *Moringa* is a deciduous, fast-growing tree which usually grows up to a height of 10-12 meters (Parrotta, 2009) and can be propagated either sexually through seeds or vegetatively through stem cuttings (Mubvuma et al., 2013). It has an open crown of fragile and drooping branches, giving the tree canopy an umbrella shape (Parrotta, 2009). The bark is white-grey in colour and has a thick cork (Parrotta, 2009).

The foliage of *Moringa* is feathery, consisting of bipinnate or tripinnate leaves, which can grow up to 45 cm long and are spirally arranged on the twigs (Parrotta, 2009). The flowers appear after six months of germination and are fragrant, bisexual, having five unequal, yellowish-white petals (Parrotta, 2009). The fruits of *Moringa* are dangling, three-sided, 20-45 cm brown pods, which mature after three months of flowering and split to release dark-brown, globular seeds of one-centimetre diameter (Palanisamy and Kumaresan, 1985). The seeds have three papery, white wings and are usually dispersed by water and wind. Trees grown from seeds usually develop deep and thick taproots with wide-spreading system of tuberous and thick lateral roots; however, trees propagated from cuttings do not develop taproots at all (Lahjie and Siebert, 1987).

2.1.2 Growing conditions

Moringa grows in tropical and sub-tropical regions of South Asia and Africa and can withstand frost and temperatures up to 48^0 C (Oliveira et al., 1999). However, *Moringa* does best in the regions where temperature ranges from 26- 40^o C and the annual rainfall is at least 500 mm (Goss, 2007). It can endure a wide range of soil types but grows best in well-drained loam to clay loam soils and neutral to slightly acidic soils as it cannot withstand prolonged water logging (Padilla et al., 2012).

2.1.3 Pharmacological potential

Moringa is often described as the 'Miracle Tree' as almost every part of the tree- leaves, flowers, pods, seeds, seed oil, bark etc.- have medicinal applications (Daba, 2016). Various parts of *Moringa* are used as circulatory and cardiac stimulants, contain antileptic, antipyretic, anti-inflammatory, anti-tumour, cholesterol lowering, antidiabetic properties, and are used for treating various diseases in indigenous medicinal systems (Anwar et al., 2007). *Moringa* is rich in phytochemicals such as tannins, alkaloids, anthocyanins, proanthocyanins, flavonoids, cinnamates and cardiac glycosides, which provide the plant its much-researched pharmacological properties (Goyal et al., 2007; Alhakmani et al., 2013). Anti-inflammatory activity of *Moringa* roots is due to the presence of aurantiamide acetate and 1,3- dibenzyl urea in the roots and root bark of the tree (Mishra et al., 2011).

In-vitro examinations of ethanolic extract of *Moringa* seeds and various extracts of its leaves showed anti-tumour activity, which is caused by isothiocyanate and thiocarbonate related compounds present in those parts of the plant (Nadkarni, 1994; Mishra et al., 2011). Strong antioxidant activity has been exhibited by aqueous, methanolic and ethanolic extracts of *Moringa* leaves, which is induced by Kaemferol (Mishra et al., 2011).

Isothiocyanate glycosides and thiocarbamate present in the ethanolic extract of *Moringa* leaves have been reported to be responsible for antihypertensive activity (Gilani et al., 1994). Aqueous, methanolic and ethanolic extracts of the leaves have been described to have anti-ulcer (Mishra et al., 2011), antiepileptic (Amrutia et al., 2011) and anti-diabetic effects respectively (Suzuki et al., 2007). Alcoholic extracts of *Moringa* seed kernels have exhibited anti-asthmatic effect (Mehta and Agrawal, 2008).

2.1.4 Food and nutrition

Moringa is the most nutritious tropical legume (Dhakar et al., 2011). Leaves of *Moringa* have high fat content, which increases their palatability (Oduro et al., 2008), and contain all essential amino acids at concentrations higher than those recommended by the Food and Agriculture Organisation (FAO) (Makkar and Becker, 1996). *Moringa* is rich in vitamins such as A, C, B complex and is also a storehouse of proteins and minerals like iron, calcium, magnesium, potassium, and zinc (Verma et al., 1976; Fuglie, 2001).

Moringa is reported to have more calcium than milk, more Vitamin A than carrots, more potassium than bananas and more iron than spinach (Fahey, 2005). Therefore, *Moringa* has a potential in combatting nutritional deficiencies and malnutrition in developing countries, especially in cases of nursing mothers and infants (Srikanth et al., 2014). The leaves of *Moringa* provide nutritional security to rural communities of developing countries when it is most needed, as the leaves of the tree appear towards the end of the dry season, when very few other sources of leafy green vegetables are available (Fisher, 2011).

Dried or fresh leaves of *Moringa* are used in various kinds of foods such as salads and porridges while the flowers are cooked and consumed as a vegetable (Lockett et al., 2000). Young pods of *Moringa* are eaten as green beans (Foidl et al., 2001) and the root bark of the

plant is used as a condiment in some tropical countries (Villafuerte and Villafuerte-Abonal, 2009).

2.1.5 Other commercial uses

Since *Moringa* is a fast-growing plant, it is planted on a large scale in India to provide wood to the paper industry. The pulp obtained from *Moringa* wood is considered suitable for paper making, wrapping, cellophane and textiles (Ganatra et al., 2012). In Jamaica, exudates of *Moringa* are used in the production of blue dye (Verma et al., 1976). A study revealed that by adding *Moringa* leaves to livestock feed, daily weight gains of livestock increased by 32% while milk production increased by 43% (Foidl et al., 2001).

Juice extracted from fresh *Moringa* leaves can act as a growth hormone and increase yields of crops by 25-35% (Foidl et al., 2001). *Moringa* offers a variety of other potential uses, such as foliar nutrient fertilizer and as green manure (Fuglie, 2001). The ben oil extracted from *Moringa* seeds is used in cooking, lubrication, and cosmetic industries (Ramachandran et al., 1980; Bosch, 2004). Seed oil of *Moringa* is also a potential contender for biodiesel production, as it meets almost all the chief specifications of biodiesel standards of Europe, U.S., and Germany (Mofijur et al., 2014).

2.1.6 Antimicrobial research

Moringa has been widely and extensively used in traditional medicines, mainly because of its antimicrobial properties. Various studies demonstrate antimicrobial properties of different parts of *Moringa*. Using disc diffusion method, fresh leaf juice of *Moringa* and aqueous extract of its seeds was found to inhibit growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria (Caceres et al., 1991). An experiment conducted in 1995

reported antifungal activity of seed and leaf extract of *Moringa* against pathogenic fungi like *Trichophyton rubrum* and *Trichophyton mentagrophytes* (Nwosu and Okafor, 1995).

N-benzyl, S-ethyl thioformate, a compound formed from chloroform extract of *Moringa*, showed antimicrobial efficacy against *S. aureus*, *Shigella dysenteriae* and *Shigella boydii* (Nikkon et al., 2003), while purified dichloromethane extract of *Moringa* capsules showed antibacterial efficacy against *Escherichia coli*, *S. aureus*, *P. aeruginosa* and *Klebsiella pneumoniae* (Nantachit, 2006). Jamil et al. (2008) studied the antibacterial and antifungal activity of seed extract of *Moringa* against microbes like *Bacillus subtilis*, *S. aureus, E. coli, Rhizopus solani* etc., and found that the growth inhibition zone showed greater sensitivity against bacterial strains as compared to fungal strains.

Antibacterial activities of ethanolic, aqueous and acetone extracts of *Moringa* leaves were found to be like antibiotics ciprofloxacin, chloramphenicol and cotrimoxazole (Doughari et al., 2007). Renitta et al. (2009) studied antibacterial activities of ethanolic extract of flowers, seeds and leaves of *Moringa* against *E. coli*, *K. pneumoniae*, *P. aeruginosa, Enterobacter, S. aureus* and *Salmonella typhi* with a standard disc diffusion method. Most of the bacteria were found to be susceptible to the ethanolic extract of *Moringa* leaves except *S. typhi*, that was found to be resistant (Renitta et al., 2009). The ethanolic extract of *Moringa* seeds was found to inhibit the growth of *S. aureus* and *K. pneumoniae*, but not the other bacteria tested. Similarly, only *K. pneumoniae* and *E. coli* were found to be susceptible to ethanolic extract of *Moringa* flowers, while the other tested bacteria were found to be resistant (Renitta et al.,2009).

Moringa oleifera cationic protein (MOCP) which is a low molecular mass protein extracted from seeds of the *Moringa* plant, has been found to be an effective coagulating agent in water treatment (Kwaambwa et al., 2010). It is responsible for inhibiting growth of coliform bacteria like *E. coli* by fusing its inner and outer membranes (Shebek et al., 2015). Root powder

of *Moringa* reduced *E. coli* colonies by 87% when added to contaminated water at 600 mg/L (Morgan et al., 2019). This makes *Moringa* root powder a promising alternative to purify contaminated water.

2.1.7 Research in water purification

Traditionally, *Moringa* seeds were used in various African countries such as Ethiopia, Uganda, and Sudan to purify contaminated water and removing its turbidity (Jahn, 1977). Seeds of *Moringa* (also referred to as 'clarifying tree' in Sudan) were crushed into a powder by Sudanese people, and then the powder was added to turbid water and stirred thoroughly to remove turbidity (Jahn, 1977).

The effectiveness of natural coagulants like *Moringa* to remove turbidity of water was found to be similar to chemical coagulants like aluminium sulphate, which are too expensive and unaffordable by developing countries (Delelegn et al., 2018). The crushed seeds of *Moringa* release dimeric cationic proteins to turbid water, which form flocculants with the water contaminants (Gassenschmidt et al., 1995). These small proteins have a positive charge and thus, attract negatively charged bacteria, viruses and other colloidal particles found such as silt and clay, allowing them to coagulate and settle (Amagloh and Benang, 2009).

Various settling times have been proposed for successful water treatment by *Moringa*. Doerr (2005) recommended 1-2 hours of settling time for all contaminants to completely settle at the bottom of container; while Lilliehook (2005) employed a settling time of 30-120 minutes for water with low, medium, and high turbidity. Mataka et al. (2006) recommended that mixture of *Moringa* seed powder and water should be kept undisturbed for five hours to attain clean drinking water as the lead levels in water decreased exponentially during the first five hours of reaction.

The seed powder of *Moringa* can flocculate various Gram-positive and Gram-negative bacteria such as *Salmonella typhimurium*, *Shigella sonnei*, *E. coli*, *Vibrio cholera*, *Streptococcus faecalis* and *Clostridium perfringens* (Madsen et al., 1987; Anwar et al., 2007). In addition to removing turbidity, this flocculation also results in decreasing the conductivity of contaminated water to $\log_{10}$ 2.34 µS/cm and increasing the pH to 7.2- 7.9, making *Moringa* seeds a non-toxic, natural, and effective alternative to purify drinking water (Amagloh and Benang, 2009). However, *Moringa* roots showed an inverse effect on conductivity and pH of contaminated water, as the roots caused significant ($p<0.05$) increase in conductivity but decreased the pH from 7.61 to 7.38 (Morgan et al., 2019).

2.2 *Zingiber officinale*

2.2.1 Botanical description

Ginger (*Zingiber officinale*) is a member of the plant family Zingiberaceae, which also includes cardamom and turmeric. It is a two to four feet tall slender monocot, perennial plant with greenish-yellow flowers (Kaufman, 2016). The leaves of ginger are 15-30 cm long, arise from sheaths wrapping the stem and are spread alternatively in two vertical rows (Kaufman, 2016). The inflorescence is solitary, in dense conelike spikes composed of overlapping green bracts, and each bract encloses a small, purple, and yellow-green flower (Aleem et al., 2020). The underground stems (known as rhizomes or roots) are thick lobed, pale yellowish and aromatic (Aleem et al., 2020).

2.2.2 Growing conditions

Ginger is native to Southeast Asia but is now cultivated in other tropical and subtropical regions such as the West Indies, Africa, Latin America, and Australia (Singletary, 2010). It requires warm and humid climate with temperature ranging between 19-28^0 C, high amount of

rainfall and relative humidity, and soil with good aeration and water holding capacity for a promising yield (Teferra et al., 2013). Ginger is sensitive to frost, water logging and salinity, but is tolerant to drought (Teferra et al., 2013).

2.2.3 Food and nutrition

Rhizomes (or roots) of ginger can be processed into syrup, powder, oleoresin, and volatile oil, and are widely used in folk medicine, as food flavourings and as dietary supplements (Bode and Dong, 2011; Gatabazi et al., 2019). Dried and ground ginger is used as a spice in South-East Asia to flavour breads, curry dishes, confections, sauces, pickles, and ginger ale, whereas fresh ginger is used in cooking as both a flavouring and a vegetable (Kaufman, 2016). In Japan, slices of ginger are consumed by people between dishes to clear the palate (Kaufman, 2016).

The rhizomes of ginger contain carbohydrates (50-70%), lipids (3-8%), terpenes, phenolic compounds, fibre, proteins, ash, amino acids, vitamins, and volatile oils (Singletary, 2010; Prasad and Tyagi, 2015). The spicy aroma of fresh ginger is mainly due to the presence of ketones— gingerols, of which [6]-gingerol is most abundant and widely studied in health-related scientific research (Bode and Dong, 2011). The pungency of dry ginger results from shogaols, which are dehydrated gingerols (Ali et al., 2008). The cultivation practices and post-harvest treatment of ginger affects the quantity and quality of biologically active constituents present in the rhizome. Similarly, the chemical components of the rhizome can differ significantly based on the geographical location of cultivation of ginger, and whether the product is dried, fresh, or processed (Ali et al., 2008).

2.2.4 Pharmacological effects

For centuries, ginger has been an essential part of traditional healing strategies in Middle East, Asia, and Europe. Even today, it is widely used in Ayurvedic, Chinese and Tibb-Unani herbal medicines for treating a wide range of ailments that include rheumatism, arthritis, sprains, muscular pains, constipation, sore throat, vomiting, fever, hypertension, and dementia (Ali et al., 2008).

Evidence primarily from in-vitro and animal studies shows that ginger can be beneficial for patients suffering from cardiovascular disease as it can counteract hyperlipidaemia, inflammation, hypertension, and platelet aggregation (Singletary, 2010). The Suekawa group studied the effect of [6]-gingerol and [6]-shogaol on blood pressure and found that the latter caused a rapid drop in bradycardia, apnoea, and blood pressure in rats (Chrubasik et al., 2005). Kadnur and Goyal (2005) reported that methanolic extract of dried ginger rhizomes caused a significant decrease in fructose-induced elevation of bodyweight, lipid levels, hyperglycaemia, and hyperinsulinemia. In-vitro studies show that aqueous extract of ginger or its pungent constituents can effectively inhibit platelet aggregation (Srivas, 1984; Koo et al., 2001). Previous studies also suggest that intake of ginger powder can significantly reduce platelet aggregation in patients suffering from coronary artery disease (Bordia et al., 1997) and can have synergistic effect with nifedipine in hypertensive patients (Young et al., 2006).

Antitumor promoter activity of ginger extracts, [6]-gingerol and [6]-paradol was demonstrated by Vimala et al. (1999) and Bode et al. (2001). There is suggestive evidence that ginger powder or an extract can alleviate the symptoms of vomiting and nausea following pregnancy, cancer therapy, surgery, and motion sickness (Chrubasik et al., 2005; Singletary, 2010). Altman and Marcussen (2001) found ginger to have moderate effect in relieving osteoarthritic pain, but patients witnessed a significant reduction in knee pain after just 6 weeks

of treatment with proprietary ginger extract. Administration of ginger was found to exert prophylactic and abortive effects in migraine headache without producing any side-effects (Mustafa and Srivastava, 1990).

2.2.5 Antimicrobial research

Hydroethanolic ginger extract has been reported to suppress the growth of various Gram-positive and Gram-negative bacteria such as *S. aureus, Haemophilus influenzae, Streptococcus pyogenes, Streptococcus pneumoniae, Salmonella typhimurium,* and *P. aeruginosa* (Chrubasik et al., 2005). Karuppiah and Rajaram (2012) reported antibacterial efficacy of ethanolic extracts of ginger rhizomes against *E. coli*, *S. aureus*, *Bacillus* sp., *P. aeruginosa* and *Proteus* sp. with the maximum zone of inhibition displayed against *Bacillus* sp. (16.55 mm) followed by *E. coli* (15.50 mm). Gupta and Ravishankar (2005) found that the commercially available ginger paste and fresh garlic paste showed better antimicrobial efficacy against *E. coli* O157:H7 than turmeric and carrot paste in ground beef and laboratory buffer. The commercial ginger paste displayed strong antibacterial activity (about 1-2 log CFU/g) in ground beef at 8^0 C, while fresh garlic paste showed similar antimicrobial efficacy (1.3 log CFU/g) against *E. coli* in buffered peptone water at the same temperature (Gupta and Ravishankar, 2005).

Gingerols and phenolic metabolites present in ginger rhizome inhibit the growth of 19 strains of *Helicobacter pylori* and enhance effectiveness of drugs combating gastrointestinal diseases caused by *H. pylori* (Mahady et al., 2003). Ginger constituents like shogaol, zingerone and essential oils have depicted antiviral and antifungal activity against *S. typhi*, *Trichophyton violaceum*, *V. cholerae* and some filamentous fungi (Jagetia et al., 2003). According to Ficker et al. (2003), [6], [8] and [10]-gingerols and [6]-gingerdiol are the chief antifungal compounds present within ginger that are active against 13 human pathogens such as *Candida albicans*,

Trichophyton mentagrophytes and *Microsporum gypseum* at <1 mg/mL concentrations. Hydroethanolic and aqueous extract of ginger can effectively inhibit the growth of *C. albicans* and *Trichomonas vaginalis* respectively (Jagetia et al., 2003). However, ginger has shown no significant action against *Aspergillus niger* and *Aspergillus flavus* (Ali et al., 2008).

Not much research has been done on reviewing ginger's potential to purify contaminated water other than its potential to inhibit growth of a variety of microorganisms that might be present in water such as *E. coli*, *P. aeruginosa*, *Salmonella* sp. etc. (Jagetia et al., 2003; Chrubasik et al., 2005; Gupta and Ravishankar, 2005; Karuppiah and Rajaram, 2012). *Moringa* and ginger root powders appear to offer a promising alternative to treat *E. coli* colonies present in contaminated water, since *Moringa oleifera* and *Zingiber officinale* grow extensively in underdeveloped and developing countries (Ethiopia, Philippines, India, etc.) where the problem of contaminated water is quite alarming. In addition to this, various parts of the plants have been reported to possess antibacterial and water purifying properties.

Chapter 3: Growing *Moringa oleifera* and *Zingiber officinale* in a greenhouse and extraction of their root powders

3.1 Abstract

Moringa oleifera (Moringa) and *Zingiber officinale* (ginger) are two tropical plants that have been extensively studied and widely used for their nutritional and medicinal properties all over the world. The objective of this study was to discuss the methods and techniques used to grow *Moringa* and ginger plants in the UNBC greenhouse, analyse their growth, and harvest and process their root powders. *Moringa* seeds were sown on two separate dates – February 27, 2019 (three seeds per pot) and March 20, 2019 (one seed per pot). The seedlings were transplanted to bigger pots in the beginning of May 2019. The height and stem diameter of the *Moringa* plants were first measured on May 27, 2019, and then on the same date of every month until their harvest in October, 2019. By the end of August, the mean height and diameter of the *Moringa* plants planted in March slightly surpassed the growth parameters of the plants planted in February. The *Moringa* plants had a significant growth over the months and variability between the *Moringa* plants was calculated to be small. A massive ginger rhizome, purchased from a supermarket, was grown in the greenhouse in 2017. In the beginning of April 2020, ginger plantlets along with their rhizomes were divided and vegetatively propagated into smaller one-litre pots. The height and stem diameter of the ginger plants was measured only once, on June 23, 2020. After growing *Moringa* and ginger plants in the greenhouse for seven months and three years respectively, their roots were harvested, dried and ground to a fine powder using Black+Decker coffee and spice grinder.

3.2 *Moringa oleifera*

3.2.1 Methods

3.2.1.1 Planting and growing Moringa in the greenhouse

Moringa seeds were obtained from Miracletree Life Science, a privately held organic farming group based in Madurai, Tamil Nadu, in southern part of India. The sun-dried *Moringa* seeds were treated with 'panchagavya' before being packed and shipped to Canada. Panchagavya is a traditional Indian pesticide and a fertilizer made from five cow products (cow dung, urine, milk, curd, ghee), jaggery, coconut and ripe bananas (Raut and Vaidya, 2018).

Moringa seeds (Figure 1) were planted in the I.K. Barber Enhanced Forestry Lab (EFL) greenhouse at University of Northern British Columbia, Prince George. A soil mixture was prepared by mixing 54 L peat, 60 L coarse sand, 69 L coir, 180 g of slow-release nutrients (21-5-6) and three tablespoons of dolomite. Using calibration buffer pouches and a pH tester, pH of this soil mixture was measured to be 4.6. One hundred *Moringa* seeds were sown manually in one-litre pots on February 27, 2019, each pot containing three seeds. After pushing the seeds about one cm deep in the soil with a finger, they were covered with soil to level the soil surface. The pots were randomly placed on the shelves of greenhouse and were given a light watering.

The greenhouse bay was kept at a temperature of 24^0 C during the day and 18^0 C during the night. The pots were given a light, frequent watering about five times a week to keep the soil consistently moist, but not wet. After 21 days of sowing, the extra seedlings from pots that showed two or more germinations (Figure 1) were transplanted to separate one-litre pots on March 20, 2019. This was done by gently digging them using a narrow trowel while trying to retain as much of the root ball as possible. Following this, there were a total of 86 pots, each

containing one healthy *Moringa* seedling. On the same day, another batch of 20 *Moringa* seeds were sown in 20 one-litre pots.

Figure 1. Indian *Moringa oleifera* seeds that were grown in UNBC greenhouse (Left). A pot with three germinating *Moringa* seedlings (Right). (*Photographs by author, taken on February 27 and March 14, 2019, respectively*).

Moringa plants received 16 hours of supplemental lighting per day from 0600 to 2200 hours using High Pressure Sodium (HPS) PL lights of 400 watts. The temperature of the greenhouse was increased to 30^0 C during the day and 24^0 C during the night at the beginning of April, after the leaves of seedlings started to curl and turn yellow. Since the pH of soil was found to be 4.6, 250 mL of a solution of 10 mL Grotek pro-silicate (0-0-3) well dissolved in 20 L water was added to each pot to increase soil pH to a value adequate for growing *Moringa*. This increased the pH of soil to 6. This solution and fertigation with Tune Up 20-10-20 Plus, a water-soluble fertilizer, was given to all plants once every week from April 5, 2019. As soon as weeds sprouted, they were removed manually and disposed in the garbage bin outside the greenhouse to prevent an outbreak.

On May 2, 2019, 74 healthy *Moringa* plants were transplanted to bigger four-litre pots as they outgrew their seed starting containers (one-litre pots). A soil mixture was prepared by mixing 90 L coarse sand, 107 L peat, 123 L coir, 321 g of slow-release nutrients and seven tablespoons of dolomite. The newly prepared soil mixture was added to the bottom of four-litre pots. In order to retain maximum root ball and to give minimum shock to the plants during transplanting, the one-litre pots were turned upside down and their rims were tapped with one hand while covering the top of the pots with the other hand to support the plants. Then, the root balls were gently taken out of the pots. The plants with their root balls were set in the four-litre pots and the soil mixture was used to cover the root ball and fill the sides of the pots. The plants were watered immediately after transplanting.

Thrips were first noticed on the undersides of leaves of several *Moringa* plants on May 6, 2019. To get rid of thrips, the plants were sprayed with a chemical insecticide, Success 480 SC on May 7, 2019 and then by another chemical insecticide– AVID (Abamectin) on May 17, 2019, when the thrips reappeared. To ensure that the HPS lights did not burn the plants or interfere with their growth, the lights were removed once the plants reached an approximate height of one to 1.5 meters.

3.2.1.2 Measuring growth of Moringa plants

The height and stem diameter of the *Moringa* plants were first measured on May 27, 2019, and then on the same date of every month until their harvest in October. Height of the plants was measured from the soil surface up to the topmost bud of the plant using a measuring tape. For measuring stem diameter of a plant, a mark on the stem was made at 3.5 cm above the soil surface by a sharpie. Using an electronic caliper, three diameter readings were taken on this mark and averaged to give stem diameter of the plant. The mean height and mean stem diameter of all *Moringa* plants were calculated from measurements taken on 27th of every

summer month– May, June, July and August. From the mean height and diameter values of the *Moringa* plants, height to diameter ratio (H:D) was calculated since it determines sturdiness, vigour, and competition in the plants (Opio et al., 2003; Haase, 2008).

One sample T-test was performed to determine if the growth of *Moringa* was significant over the months. For this, monthly height measurements of all the *Moringa* plants were taken as a sample and were compared to the mean height of the previous month to calculate t- and p-values of height in each month. Similar calculations were performed to compute t- and p-values of stem diameter of the *Moringa* plants during the summer months. One sample T-test was performed using R Studio software and significance of the test was determined at a p-value of 0.05.

3.2.1.3 Harvesting and processing of Moringa roots

After about seven months of growing *Moringa* in the greenhouse, roots of half of the plants planted on February 27, 2019, were harvested on 4 October (Figure 2) and the remaining half were harvested on October 8, 2019. The roots of *Moringa* plants sown later in March were harvested in mid-October, on October 18, 2019. The roots were harvested by first cutting the stem of plants at soil surface using a garden lopper, the pots were then inverted, and their rims were tapped until their root balls dropped (Figure 2). The soil was brushed off to expose as much of the roots as possible. Pruning shears were used to cut distinctive clumps of roots by removing thin root ends or remaining trunk base.

The harvested *Moringa* roots were gently washed with water and their root barks peeled off using a stainless-steel vegetable peeler. The peeled roots of each *Moringa* plant were packed in separate air-tight plastic bags with inscribed plant number, its date of sowing, transplanting and harvest. These airtight bags were stored in the -18^{o} C freezer of Northern Analytical Laboratory Services (NALS).

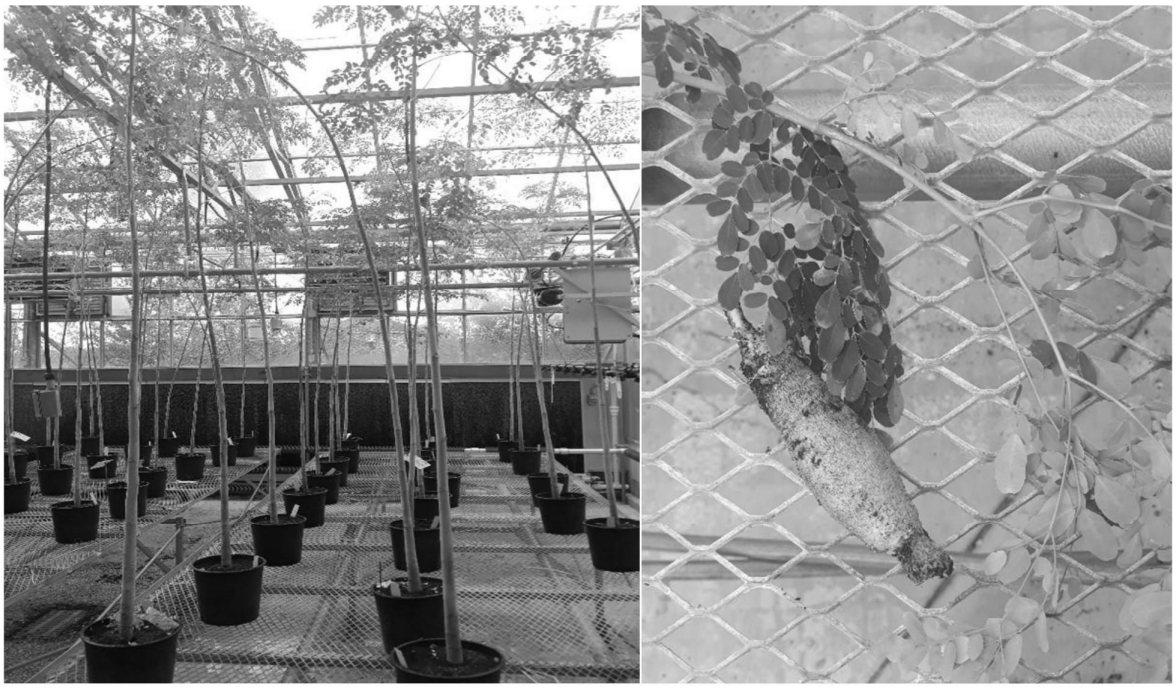

Figure 2. The *Moringa* plants on the day of their harvesting (Left). A harvested *Moringa* root (Right). (*Photographs by author, taken on October 4, 2019*).

3.2.1.4 Extraction of dried Moringa root powder

For generating simple random samples, sixteen *Moringa* root samples were arbitrarily selected for freeze-drying at the beginning of June 2020. Three of these root samples were of the *Moringa* plants sown later in March, while the other 13 root samples were of the plants planted in February 2019. This was done to ensure proper representation of *Moringa* plants from both planting dates in the selected samples.

Excess ice from the airtight containers was dumped before placing the bags in the drying chamber of FreeZone^{6} freeze dryer by Labconco^{TM}. Once the temperature within the drying chamber dropped to -40^{0} C, the vacuum pump was automatically activated. In about ten minutes, the vacuum level within the drying chamber stabilized to 0.420 mBar and the temperature dropped further down to -44^{0} C. The samples were left in the freeze dryer for two days.

After two days, *Moringa* root samples were taken out of the freeze dryer, placed on a wooden chopping board and were cut into smaller fragments using a plastic knife. These root fragments were then ground into a fine powder using commercially available, electric Black+Decker coffee and spice grinder. The stainless-steel bowl and blades of the grinder, the knife and the chopping board were all cleaned with distilled water and dried with KimTech Kimwipes before their use. The grinder was run for about a minute until the roots were milled into a fine powder. The finely ground *Moringa* root powders were sealed in separate airtight plastic bags (root powders of each plant in a separate bag) that were then stored in -18^0 C freezer at NALS.

3.2.2 Results

3.2.2.1 Planting and growing Moringa in the greenhouse

The *Moringa* seeds sown on February 27, 2019, showed 86% germination while the seeds sown on March 20, 2019, showed 90% germination.

3.2.2.2 Measuring growth of Moringa plants

As stated in section 3.1.1.2 of the thesis, the mean height and mean stem diameter of all *Moringa* plants were calculated from measurements taken on 27^{th} of every summer month– May, June, July, and August. Figures 3 and 4 compare the mean height and stem diameter of *Moringa* plants planted on February 27, 2019, and March 20, 2019. In the month of August, the mean height and diameter of *Moringa* plants planted on March 20 surpassed the mean height and diameter of the plants grown on February 27, 2019 (Figure 3 and 4).

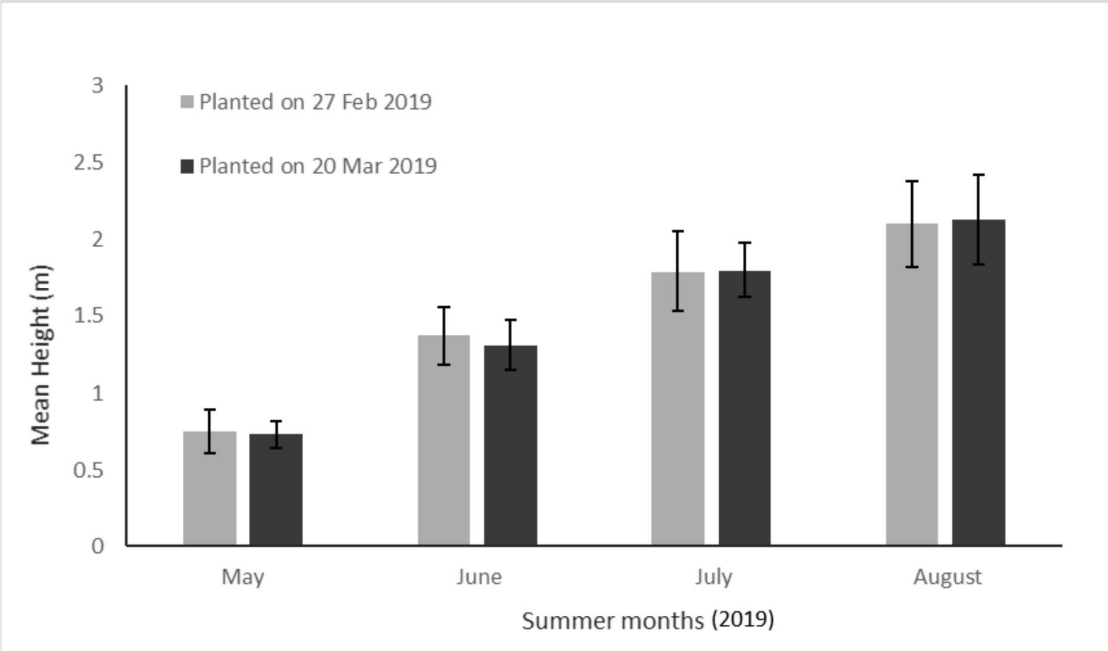

Figure 3. Growth in height (m) of *Moringa* over summer months in 2019, showing standard deviation as error bars.

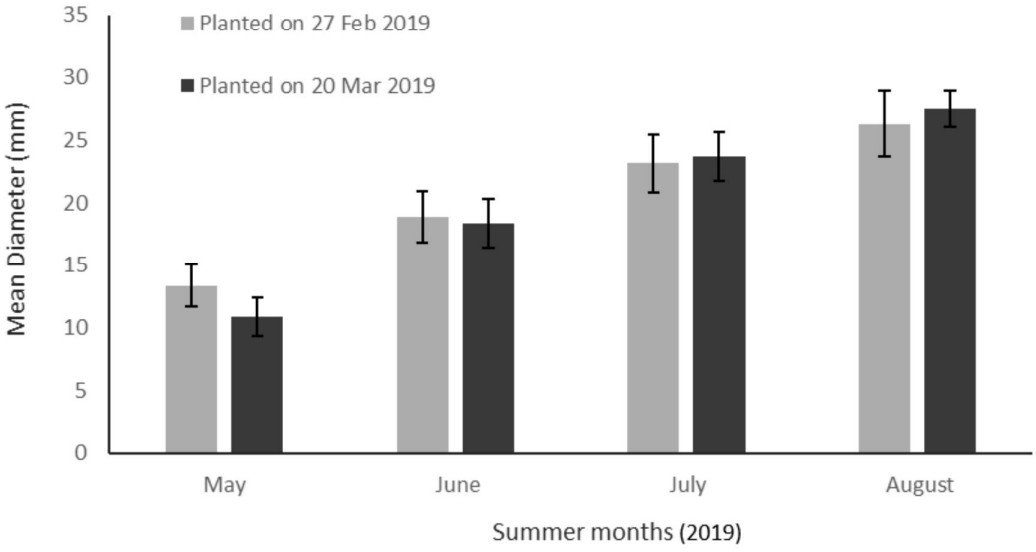

Figure 4. Diameter growth (mm) in *Moringa* over summer months in 2019, showing standard deviation as error bars.

Height to diameter ratio (H:D) of *Moringa* plants in the months of May, June, July and August are shown in Table 1. A significant (p-value <0.05) increase in H:D was detected in *Moringa* plants sown on February 27, over the summer months, however, the H:D ratios of plants sown later on March 20, did not observe a significant increase over the summer months.

Table 1. Height to diameter ratio (and standard deviation) of *Moringa* plants sown on two separate dates –February 27, 2019, and March 20, 2019, over the summer months.

	Plants sown on 27 February 2019	**Plants sown on 20 March 2019**
Month	H:D ratio	H:D ratio
May	56.94 (10.55)	67.41 (3.88)
June	73.23 (9.99)	71.99 (9.17)
July	77.42 (10.01)	75.87 (5.47)
August	80.14 (9.46)	77.47 (10.19)

In order to determine if the growth of *Moringa* was significant over the months, one sample T-test was performed using R Studio software. The t- and p-values of height and stem diameter of the *Moringa* plants during the summer months have been listed in Table 2. T-test was performed by taking the raw values of the observation month and comparing them to the mean of the previous month. All the p-values of height and stem diameter of *Moringa* plants were found to be less than 0.05 (Table 2).

Table 2. Determination of significant differences in height and diameter growth in *Moringa* plants over the summer months.

		Plants sown on February 27		**Plants sown on March 20**	
Variable	Month	t- value	p-value	t-value	p-value
Height	June	24.64	<0.001	12.15	<0.001
(m)	July	7.83	<0.001	8.94	<0.001
	August	8.62	<0.001	3.74	0.002
Diameter	June	20.16	<0.001	12.64	<0.001
(mm)	July	14.21	<0.001	8.97	<0.001
	August	9.21	<0.001	8.66	<0.001

3.2.3 Discussion

On drawing comparison between the mean height and mean stem diameter of *Moringa* plants planted on February 27, 2019 and March 20, 2019, it was noticed that the mean measurements of the latter slightly surpassed those of the plants planted earlier in February (Figure 3 and 4). This might be because the seeds sown later in March were sown one in each pot, had sufficient growing space and thus, the seedlings did not have to compete for light, water and nutrients with other seedlings in the same pot (Milla et al., 2009) and also did not have to undergo the shock of first transplanting. This luxurious consumption of water, nutrients and light by *Moringa* seeds planted on 20 March could also be the reason behind their greater germination rate than the seeds planted on 27 February, and their non-significant (p-value >0.05) increase in H:D ratios. The H:D ratio of *Moringa* plants increased over the summer months (Table 1), thus indicating an increasing competition between the plants for light and/or availability of nutrients and moisture (Opio et al., 2003). Since all the p-values of height and stem diameter of *Moringa* plants were found to be less than 0.05 (Table 2), it meant that all the *Moringa* plants had a significant growth over the months, regardless of their planting dates.

The size of standard deviation bars was small in all the cases and the data lied within one standard deviation of the mean (Figure 3 and 4). This meant that all the height and diameter values were closely spread around their respective mean values, which told us that there was only minor variability between all the *Moringa* plants. In addition to this, all the plants received similar treatment in the greenhouse. A small and insignificant variability between the *Moringa* plants meant that their root powders could be mixed before performing various microbial and chemical tests in the lab. Therefore, all *Moringa* root powder samples were added to a 1-gallon Ziploc bag and shaken vigorously for about two minutes until all the samples were thoroughly mixed to form a homogeneous, composite *Moringa* root powder sample.

3.3 *Zingiber officinale*

3.3.1 Methods

3.3.1.1 Planting and growing ginger in the greenhouse

Ginger was purchased from a supermarket and a single, massive rhizome was grown in I.K. Barber Enhanced Forestry Lab (EFL) greenhouse at UNBC in 2017. UNBC's Standard Potting Mix, which is prepared by mixing three bales of peat, three 20 L buckets of sand, three 20 L buckets of perlite, three 20 L buckets of vermiculture, two cups of Micromax micronutrients and three litres of 14-14-14 slow-release fertilizer, was used for growing ginger in the greenhouse. The rhizome was grown about an inch deep in the soil in a single Lisbo 380X300 mm pot.

The pot was kept at a temperature of 20^0 C during the day and 16^0 C during the night. In the winter months, they received 10 hours of supplemental lighting per day from 0900 to 1900 hours using High Pressure Sodium (HPS) PL lights of 400 watts. However, in the summers (2017-2020), no supplemental lighting was given. Ginger received weekly fertilization with a 20-20-20 ratio fertilizer and was watered when the pot soil appeared dry. Typically, ginger received watering three times a week in the summers and about two times a week in the winters. On appearance of mites and whitefly, the ginger plantlets were sprayed with Avid (abamectin) on December 18 and 28, 2018, and then with Talus (buprofezin) and Avid 1.9% EC on March 1 and 18, 2019 respectively. On February 14 and 21, 2020, the plant was sprayed with Orthene 75% (acephate 75%) and Enstar EW (kinoprene 18.42%) to counter the attack of mealybugs and aphids respectively.

In the beginning of April 2020, ginger plantlets along with their rhizomes were divided and vegetatively propagated into ten smaller one-litre pots. These pots received

treatment similar to the previous crop— 16-20^0 C temperatures, watering about 3 times each week and regular fertilization with 20-20-20. To impede the attack of various insects, the ginger plants were sprayed with Kontos (spirotetramat), Success 480 SC (Spinosad), Pylon (chlorfenapyr) and Ventigra (afidopyropen) on 16 April, 23 April, 15 May, and June 2, 2020 respectively.

3.3.1.2 Measuring growth of ginger plants

The height and stem diameter of the ginger plants was measured on June 23, 2020, before harvesting the plants. Heights of the plantlets were measured from the soil surface to their topmost bud using a measuring tape. Average heights of all the plantlets growing within a pot was calculated. Similarly, stem diameter of each plantlet was measured 2.5 cm above the soil surface using an electronic caliper and average stem diameter of all plantlets within a pot was calculated and regarded as the mean stem diameter of that plant.

3.3.1.3 Harvesting and extraction of ginger powder

Ginger plantlets were harvested on June 23, 2020. To procure the ginger rhizomes, stems of the plantlets were cut at the soil surface using pruning shears. Then, the soil was moved towards the edges of each pot to expose the rhizomes. The rhizomes were removed from the pots and adhering soil was brushed off with hands. Roots and residual stem base were detached from the rhizomes using pruning shears.

The rhizomes were gently washed with water and were peeled using a stainless-steel vegetable peeler. The washed, peeled rhizomes were packed in air-tight bags with inscribed plant number. These air-tight bags were placed in the NALS freezer at -18^0 C for two days. The ginger roots were freeze dried and ground into powder using the same methods and techniques used to process *Moringa* roots (Figure 5).

Figure 5. Grinding of harvested and freeze-dried roots of a ginger plant using Black+Decker coffee and spice grinder. (*Photograph by author, taken on July 6, 2020*).

3.3.2 Results

The height (expressed in cm) and stem diameter (in mm) of the ten ginger plants, measured on June 23, 2020, have been provided in Table 3. Height to diameter ratio (H:D) of each ginger plant has also been included in Table 3.

Table 3. The average height (in cm), stem diameter (in mm), and height to diameter ratio (H:D ratio) of the ten ginger plants.

Plant number	Average height (cm)	Average diameter (mm)	Height: Diameter (H:D)
1	36.5	7.37	49.53
2	41.6	8.99	46.27
3	37.5	8.21	45.68
4	48.66	9.77	49.81
5	39.5	8.28	47.71
6	26.5	5.72	46.33
7	36.66	6.82	53.75
8	34	6.14	55.38
9	47	8.09	58.10
10	29	5.85	49.57

3.3.3 Discussion

Previous literature on height, stem diameter and H:D ratio of three-year-old ginger plants could not be found and hence, the growth parameters of the ginger plants (Table 3) could not be compared with previous studies. As the height, diameter, and H:D ratio of the ginger plants was measured only once (on the day of their harvesting), a pronouncement regarding their growth patterns over months, and increasing or decreasing competition between the plants could not be made. Future studies should document growth of ginger plants over months (like growth analysis of *Moringa* plants detailed in section 3.1 of the thesis). This would help to detect any competition between the plants, to determine if their growth over months is significant or not, and finally, to calculate variability between the ginger plants.

3.4 Conclusion

Since all the ginger plants were vegetatively propagated from a single ginger rhizome and all the plants received similar treatment in the greenhouse, the variability between their roots was assumed as small and insignificant. Similarly, the *Moringa* plants were found to have minor variabilities between them (section 3.2.3 of the thesis). Hence, the ground *Moringa* and ginger root powders were mixed to form composite samples before performing chemical analysis and various microbial tests in the lab.

Chapter 4: Chemical analysis of *Moringa* and ginger root powders

4.1 Abstract

Moringa (*Moringa oleifera)* and ginger (*Zingiber officinale*) have been extensively studied and widely used for their nutritional and medicinal properties all over the world. The objective of this study was to conduct a complete chemical analysis of the root powders extracted from roots of *Moringa* and ginger plants grown in the UNBC greenhouse. The most abundant metal found in both root powders through Inductively coupled plasma- optical emission spectrometry (ICP-OES) was potassium, with a mean concentration of 6,066.5 mg/kg in *Moringa* root powder and 17,484.33 mg/kg in ginger powder. Calcium, magnesium, sulphur, sodium, and phosphorus were other metals present in the roots in high concentrations. Harmful, heavy metals such as lead, mercury, arsenic and cadmium were least abundant in the root powders, with concentrations less than 10 mg/kg. Using Elemental Analysis method, total carbon and nitrogen found in *Moringa* root powder was 42.23% (by oven-dry mass) and 0.25% respectively, and in ginger powder was 41.90% and 3.7% respectively. Results of Ion Chromatography revealed that concentrations of anions like sulphate, phosphate, chloride, and nitrate were higher in ginger than in *Moringa* root powder. This explains the higher Electrical Conductivity (EC) of ginger (12.45 mS/cm) compared to *Moringa* root powder, that had 4.74 mS/cm. Both root powders were slightly acidic with pH values ranging from 5 to 6. The abundance of essential elements and anions, and the scarcity of toxic, heavy metals in the two root powders justifies their use as a source of food for humans and as a point-of-use water treatment method.

4.2 Introduction

Moringa (*Moringa oleifera*) and ginger (*Zingiber officinale*) have been an integral part of Asia's folk medicine for years. Currently, these tropical plants are being extensively studied and widely used for their nutritional and medicinal properties all over the world.

Various parts of *Moringa* tree are used as circulatory and cardiac stimulants, and are known to contain antipyretic, antileptic, anti-inflammatory, anti-tumour, cholesterol lowering and antidiabetic properties (Anwar et al., 2007). *Moringa* is rich in phytochemicals such as alkaloids, tannins, anthocyanins, flavonoids, proanthocyanins, cinnamates and cardiac glycosides that provide the plant its pharmacological properties (Goyal et al., 2007; Alhakmani et al., 2013). *Moringa* roots are used to treat inflammation, rheumatism, articular pain, lower back pain, constipation (Anwar et al., 2007) and have anti-spasmodic, anti-tumour and antimicrobial properties (Caceres et al., 1991; Bose, 2007; Mishra et al., 2011). Various extracts of *Moringa* roots showed presence of saponins, carbohydrates, cardiac glycosides, steroids, terpenes, alkaloids, and flavonoids (Furo and Ambali, 2012). Phytochemicals such as 4-O-(α-l-rhamnopyranosyloxy)-benzylglucosinolate (glucomoringin), benzylglucosinolate (glucotropaeolin) and 3.57- 4.38% crude protein have also been detected in *Moringa* roots (Amaglo et al., 2010).

Unlike organic components, inorganic elemental components of *Moringa* roots have not been extensively researched (Morgan et al., 2019). Amaglo et al. (2010) detected presence of <0.1% (w/v) sodium, 2.05% (w/v) potassium and 0.3% (w/v) calcium in *Moringa* roots. The first complete analysis of all the inorganic components of *Moringa* roots was done by Morgan et al. (2019). Since metal uptake in plants is affected by various factors (soil pH, metal concentrations in soil, organic matter content, cation exchange capacity etc.), there was a need to chemically analyse *Moringa* and ginger root powders before studying their antibacterial

efficacy against *E. coli*. In addition to this, there were significant differences in the handling, processing, and storage of *Moringa* roots from the methodology used by Morgan et al. (2019).

Pre- and post-harvest handling of the *Moringa* plants, such as types of pesticides used, the procedure of root drying, processing, and storage, in this research varied greatly from Morgan (2019). Safer's Soap and Enstar were used by Morgan et al. (2019) to treat thrips and whiteflies that appeared on *Moringa* plants, while Success 480 SC and AVID (Abamectin) were used on the appearance of thrips in this research. Morgan et al. (2019) oven-dried the *Moringa* roots at 70^0 C for five days, whereas in this research, the roots were freeze-dried for two days (read section 3.1.4 for more details on root processing). The dried *Moringa* roots were ground using a ball grinder and then the root powder was stored at room temperature in an airtight jar by Morgan et al. (2019). But in this research, the roots were ground using Black+Decker coffee and spice grinder and were stored at -18^0 C.

Ginger is known to treat a wide range of ailments that include rheumatism, arthritis, sprains, muscular pains, constipation, sore throat, vomiting, fever, hypertension, and dementia (Ali et al., 2008). It has been found to contain antioxidant, antimicrobial, and anti-inflammatory properties. Ginger is rich in bioactive compounds such as gingerols, paradols and shogaols that provide the plant its much-researched medicinal properties (Mao et al., 2019).

Like *Moringa* root, not much research has been done to determine the concentrations of non-essential and essential metals present in ginger, except Wagesho and Chandravanshi (2015). They found the mean range (µg/g dry weight basis) of various metals found in ginger to be- Calcium (2000–2540), Magnesium (2700–4090), Iron (41.8–89.0), Zinc (38.5–55.2), Copper (1.1–4.8), Cobalt (2.0–7.6), Chromium (6.0–10.8), Manganese (184–401), Nickel (5.6–8.4) and Cadmium (0.38–0.97).

The chemical metallic and non-metallic elements present in *Moringa* and ginger root powders could affect their viability as a point-of-use water treatment (Morgan et al., 2019). Presence of beneficial elements such as calcium and iron in the root powders would offer additional benefits of providing nutrients in water, but detection of heavy metals like lead, mercury, or toxic anions like fluoride in high amounts would render the root powders unsuitable for water treatment and consequently unsafe for ingestion.

Concentrations of total carbon and nitrogen (and subsequently C:N ratio) present in *Moringa* and ginger root powders were also measured because occurrence of these two essential nutrients in their roots in adequate amounts would be beneficial for human health as carbon and nitrogen are the two most essential and vital elements of the human body. The concentrations of these two elements in *Moringa* and ginger root powders have not been previously determined by any study. In addition, physical parameters such as pH and electrical conductivity of *Moringa* and ginger root powders were measured as they are important indicators of a plant's health that impact mineral nutrition and water absorption in the plants. An adequate pH of the root powders would ensure that they are safe for human consumption. A complete chemical analysis of the root powders would also help the future studies to replicate the antimicrobial tests and obtain consistent results by using *Moringa* and ginger root powders with similar chemical composition.

The objectives of this chapter were to determine: (1) concentrations of metals present in *Moringa* and ginger root powders by ICP-OES (Inductively coupled plasma-optical emission spectrometry); (2) total carbon and nitrogen present in the root powders by Elemental Analysis; (3) concentration of anions by Ion Chromatography; and (4) electrical conductivity and pH of the *Moringa* and ginger root powders.

4.3 Methods

4.3.1 Determining particle size of Moringa and ginger root powders

Malvern Mastersizer 3000 with Hydro LV module was used to determine particle size of dried *Moringa* and ginger root powders. Mastersizer 3000 (by Malvern) uses the laser diffraction technique to measure particle size of samples, i.e., it measures the intensity of light that gets scattered when a laser beam passes through a dispersed particulate sample.

Small samples of *Moringa* and ginger root powders were successively dispersed into Hydro LV unit to produce stable and completely dispersed suspensions. The samples were run between 2% and 20% obscuration at 2100 rpm stirrer speed and were each sonicated for 19 seconds prior to their run. The samples were run as per the methods mentioned in Malvern Mastersizer 3000 instrumentation (Malvern© Instruments Ltd., 2013). The particles of both the samples were modelled with "Wood Flour" using Refractive Index 1.53. Both *Moringa* and ginger root powder samples ran as 5 replicates.

4.3.2 Determining metal concentrations in ginger and Moringa root powders

The ground and homogenised samples of *Moringa* and ginger roots were dried at 55^0 C for 48 hours. Four replicates of *Moringa* root powder sample and three replicates of ginger powder sample were weighed to 0.2 grams, and block digested in 15 mL digestion tubes for 2.5 hours (45-minute ramp to 95^0 C). Trace Metal Grade and strong mineral acids—nitric acid and hydrochloric acid, were used for digestion as these acids can determine lowest contaminant levels (Caledon Laboratories, 2020). The sample replicates were then diluted to 15 mL with Type 1 water (18.2MΩ) and run on ICP-OES (Agilent Technologies 5100 ICPOES) to determine concentrations of metals present in *Moringa* and ginger root powders.

4.3.3 Determining total carbon and nitrogen present in Moringa and ginger root powders

A Costech Elemental Combustion System (ECS) 4010 CHNSO analyser was used to determine percent by oven-dry mass of total carbon and nitrogen present in the two root powders. Four replicates of *Moringa* root powder sample and three replicates of ginger root powder sample were weighed out to 0.5 grams each in tin capsules.

The samples were combusted in ECS 4010 CHNSO analyser through standard sequential combustion/ reduction setup recommended by Costech in ECS 4010 brochure (Costech© Analytical Technologies Inc., 2005). Helium 5.0 with a flowrate of 100 mL per minute was employed as a carrier gas to bring combustion gases to a gas chromatographic (GC) separation column and thermal conductivity detector. The combustion oven was set to 1000^0 C, the reduction oven to 650^0 C and the GC oven to 45^0 C. Gases were then separated on a standard 2 m (Costech 051081) column and quantified with the built-in thermal conductivity detector (TCD).

4.3.4 Determining concentrations of anions in Moringa and ginger root powders

The procedure from United States Environmental Protection Agency (EPA) Method 300.0 was followed for determining concentration of various anions present in *Moringa* and ginger root powders (Pfaff, 1993). Four replicates of dried *Moringa* root powder and three replicates of dried ginger powder were weighed to one gram and added to 15 mL tubes. To each of these tubes, 10 mL (Liquid: Solid ratio= 10:1) of Type 1 water (18.2MΩ) was added. The tubes were capped and shaken at 80 rpm in end-over-end rotating shaker for 10 minutes, following which they were centrifuged at 3000 rpm for five minutes using HERMLE Z382K (by Hermle Labortechnic GmbH). The contents of the tubes were filtered through 0.45 µm syringe filters and then analysed on Dionex ICS-5000 for determining concentrations of

fluoride, chloride, nitrite, sulphate, bromide, nitrate and phosphate in *Moringa* and ginger root powders.

4.3.5 Determining electrical conductivity and pH of the root powders

Four replicates of dried *Moringa* root powder sample and three replicates of dried ginger root powder sample were weighed to 10 grams and added to 50 mL tubes. To each of these tubes, 40 mL of MilliQ water (Liquid: Solid ratio= 4:1) was added. The tubes were capped and shaken at 80 rpm in end-over-end rotating shaker for 60 minutes, after which they were centrifuged at 3000 rpm for 10 minutes and then decanted. The sample replicates were centrifuged for double the amount of time (10 minutes) than the time taken for centrifugation in ion chromatography (5 minutes). This was done to provide sediments enough time to settle as it was followed by decantation, and not filtration by syringe filters (as in case of ion chromatography). Electrical conductivity and pH of the root sample replicates was determined by using OAKTON CON700 (OAKTON® Instruments, 2010) and Orion 420A+ (Orion Aplus™, 2003) respectively.

4.3.6 Statistical analysis

R Studio software was used to calculate the mean electrical conductivity, pH, concentrations of metals, non-metallic elements, and anions of the four replicates of *Moringa* root powder and three replicates of ginger powder. Standard deviations from the mean values were also calculated using R Studio software.

4.4 Results

4.4.1 Particle size of Moringa and ginger root powders

Table 4 compares the mean standard percentile readings of *Moringa* and ginger root powders. Dv 10, Dv 50 and Dv 90 show the maximum particle diameter (in µm) below which 10%, 50% and 90% of sample volume exists (here 'D' and 'v' stand for diameter and volume distribution). Particle size of 90% of the *Moringa* and ginger root powders was under 438 and 364 µm respectively and the most reoccurring particle sizes in the two root powders (mode) were 22 and 176 µm respectively (Table 4).

Table 4. Mean percentile readings (and standard deviations) of particle size of *Moringa* and ginger root powders.

	***Moringa* root powder**	**Ginger root powder**
Volume distribution	Mean percentile	Mean percentile
Dv 10 (µm)	13.0 (0.1)	9.29 (0.03)
Dv 50 (µm)	53.9 (1.8)	73.4 (1.1)
Dv 90 (µm)	438 (16)	364 (2)
Mode (µm)	22 (n/a*)	176 (5)

*Sample mode was identical for all five replicates, resulting in no standard deviation.

Table 5 shows the distribution of particle sizes of *Moringa* and ginger root powders into five size classes. About 48% of the *Moringa* root powder had particle size between 2 to 50 µm, whereas about 50% of the ginger root powder had particle size between 50 to 500 µm.

Table 5. Mean distribution (and standard deviation) of *Moringa* and ginger root powders into different size classes.

	***Moringa* root powder**	**Ginger root powder**
Particle size class	Mean percentage	Mean percentage
<2 µm	0.91 (0.01)	1.05 (0.01)
2- 50 µm	47.9 (0.5)	43.3 (0.2)
50- 500 µm	43.5 (0.6)	50.9 (0.4)
500- 1000 µm	7.3 (0.4)	4.6 (0.2)
>1000 µm	0.32 (0.33)	0.13 (0.06)

4.4.2 Concentrations of metals present in Moringa and ginger root powders

Inductively coupled plasma- optical emission spectrometry (ICP-OES) was used to measure concentrations of 27 metals in dried, powdered roots of *Moringa* and ginger. The metals to be tested were chosen in a broad sweep, keeping in mind the list of metals used by Northern Health, British Columbia, to analyse their chemical samples and the metals outlined in Northern Health Guidelines for the approval of Waterworks (Northern Health, 2020). Table 6 shows the mean concentrations of the 27 metals and the standard deviation found in *Moringa* and ginger root powders. The mean concentrations of the metals were expressed in mg/kg.

The most abundant metal found in *Moringa* roots was potassium, which was present in a mean concentration of 6,066.5 mg/kg (Table 6). The *Moringa* roots had high mean concentrations (>1,000 mg/kg) of calcium, magnesium and sulphur, and moderate concentrations (>700 mg/kg) of sodium and phosphorus. Barium was present in the *Moringa* roots with a mean value of 10.99 mg/kg. There were less than 10 mg/kg of aluminium, arsenic, boron, iron, mercury, manganese, molybdenum, lead, antimony, selenium, tin, uranium and zinc. Cadmium, cobalt, chromium, copper, nickel, vanadium and zirconium were the least abundant of the measured metals with values below 1 mg/kg in the *Moringa* roots.

In ginger root powder, potassium was the most abundant metal found, with a mean concentration of 17,484.33 mg/kg (Table 6). The ginger root powder had high mean concentrations (>1,000 mg/kg) of magnesium, sodium, phosphorus and sulphur, and moderate mean concentration (>700 mg/kg) of calcium. Barium, iron, manganese and zinc were all present in the ginger roots with mean values between 10 and 500 mg/kg. There were less than 10 mg/kg of aluminium, arsenic, boron, copper, mercury, nickel, molybdenum, lead, antimony, selenium, tin and uranium in the ginger roots. Cadmium, cobalt, chromium, vanadium and zirconium were the least abundant metals present in ginger roots with values below 1 mg/kg.

Table 6. Mean concentrations (and standard deviations) of various metals found in *Moringa* and ginger root powders.

Metals	*Moringa* root powder (mg/kg)	Ginger powder (mg/kg)
Aluminium	<8.25* (0)	<8.25* (0)
Arsenic	<6.75* (0)	<6.75* (0)
Boron	4.31 (0.07)	7.06 (0.09)
Barium	10.99 (0.13)	17.89 (0.23)
Calcium	1,810.25 (19.99)	966.33 (6.66)
Cadmium	<0.23* (0)	0.34 (0.07)
Cobalt	<0.75* (0)	<0.75* (0)
Chromium	<0.23* (0)	<0.26* (0.04)
Copper	<0.77* (0.03)	8.64 (0.23)
Iron	6.88 (0.28)	77.47 (0.25)
Mercury	<3.3* (0)	<3.3* (0)
Potassium	6,066.5 (57.72)	17,484.33 (112.93)
Magnesium	1,183.5 (12.15)	4,414.33 (30.62)
Manganese	9.26 (0.05)	460.81 (5.44)
Molybdenum	<1.05* (0)	<1.07* (0.02)
Sodium	878.75 (5.74)	1,916 (4)
Nickel	<0.83* (0.11)	1.3 (0.09)
Phosphorus	986 (11.80)	6,417.67 (38.79)
Lead	<2.25* (0)	<2.25* (0)
Sulphur	1,232.5 (18.36)	3,977.67 (37.01)
Antimony	<4.1* (0)	<4.1* (0)
Selenium	<9* (0)	<9* (0)
Tin	<6.8* (0)	<6.8* (0)
Uranium	<4* (0)	<4* (0)
Vanadium	<0.75* (0)	<0.75* (0)
Zinc	5.21 (0.10)	46.69 (0.48)
Zirconium	<0.3* (0)	<0.3* (0)

*The metals with mean values marked with ‘less than’ (<) sign have concentrations below the method detection limit.

Both ginger and *Moringa* root powders were most abundant in potassium and least abundant in cadmium, cobalt, chromium, vanadium, and zirconium (Table 6). However, a direct comparison between the concentration of metals found in the root powders cannot be made as the *Moringa* and ginger roots were harvested at different growth periods. *Moringa* roots were harvested after about seven months of growing *Moringa* plants in the greenhouse, whereas ginger was harvested after about three months of transplanting the rhizomes, that were previously grown for three years.

4.4.3 Total carbon and nitrogen present in Moringa and ginger root powders

Elemental analysis (EA) was done to determine the amount of two essential inorganic, non-metallic elements— carbon and nitrogen, present in *Moringa* and ginger root powders. The mean percent by oven dry mass of the total carbon and nitrogen present in *Moringa* and ginger root powder replicates are given in Table 7. The Carbon: Nitrogen ratio (C: N) in *Moringa* root powder was found to be 168.92, whereas in ginger powder, it was found to be 11.32.

Table 7. Means (and standard deviations) of total carbon and nitrogen detected in *Moringa* and ginger root powders.

Total carbon and nitrogen	*Moringa* root powder (%)	Ginger root powder (%)
Total carbon	42.23 (0.20)	41.90 (0.06)
Total nitrogen	0.25 (0.01)	3.7 (0.07)

4.4.4 Concentrations of anions present in Moringa and ginger root powders

Ion chromatography was conducted to determine concentrations of inorganic anions present in *Moringa* and ginger root powders. The seven anions mentioned in 'Part A' of EPA Method 300.0 (specified by Environmental Protection Agency of U.S.) were chosen to be

analysed for this chemical analysis. These anions are the most commonly investigated anions while undergoing analysis of drinking water, groundwater, wastewater and solids (Pfaff, 1993). Table 8 gives the mean concentrations of the seven anions and the standard deviation found in *Moringa* and ginger root powder replicates.

Table 8. Mean concentrations (and standard deviations) of selected anions found in *Moringa* and ginger root powders.

Anions	*Moringa* root powder (mg/kg)	Ginger root powder (mg/kg)
Fluoride	178 (1.16)	182.77 (2.31)
Chloride	454.25 (2.22)	737.33 (7.23)
Nitrite	<10* (0)	<10* (0)
Sulphate	6,035 (334.18)	9,511.33 (46.46)
Bromide	<10* (0)	<10* (0)
Nitrate	53 (0.82)	9,326 (187.74)
Phosphate	1,029.25 (17.02)	12,653.67 (1494.94)

*The anions with mean values marked with 'less than' (<) sign have concentrations below the method detection limit.

Sulphate was the most abundant anion in *Moringa* roots with mean concentration of 6,035 mg/kg (Table 8). The roots had high mean concentration (>1,000 mg/kg) of phosphate and moderate concentrations (>100 mg/kg) of chloride and fluoride. Nitrate was present in *Moringa* roots with a mean value of 53 mg/kg. Nitrite and bromide were the least abundant anions present in the roots with concentrations lower than 10 mg/kg (Table 8).

In ginger root powder, the most abundant inorganic anion was phosphate, with a mean concentration of 12,653.67 mg/kg. Sulphate and nitrate were also present in the ginger roots in high amounts, with mean concentrations higher than 9,000 mg/kg (Table 8). The ginger roots had moderate concentration (>700 mg/kg) of chloride and above 100 mg/kg concentration of fluoride. Like *Moringa* root powder, ginger powder was least abundant in bromide and nitrite, their concentrations being lower than 10 mg/kg (Table 8).

4.4.5 Electrical conductivity and pH of Moringa and ginger root powders

The mean EC and pH (calculated from values of respective replicates) of ginger and *Moringa* root powder are given in Table 9. Ginger powder had 7.71 mS/cm higher electrical conductivity than *Moringa* root powder (Table 9). Both the root powders were slightly acidic with pH values lying between 5 and 6 (Table 9).

Table 9. Means (and standard deviations) of electrical conductivity and pH of the *Moringa* and ginger root powders.

Electrical conductivity and pH	Units	*Moringa* root powder	Ginger root powder
Electrical conductivity	mS/cm	4.74 (0.01)	12.45 (0.33)
pH	-	5.33 (0.01)	5.75 (0.01)

4.5 Discussion

4.5.1 Particle size of Moringa and ginger root powders

Particle size of *Moringa* and ginger root powders was measured to ensure that the root powders were ground to such a fine powder that their respective particles would be filtered out by Whatman 150 mm, grade 2 filter paper before performing antibacterial tests. Whatman grade 2 filter paper withholds all the particles in the size range of 8 µm and more. Since the most reoccurring particle sizes in *Moringa* and ginger root powders were 22 and 176 µm respectively (Table 4), this meant that Whatman grade 2 filter paper would withhold only *Moringa* and ginger root particles but would allow *E. coli* to pass through, as the average size of *E. coli* is known to fall between 1-3 µm (Reshes et al., 2008).

4.5.2 Concentrations of metals found in Moringa and ginger root powders

The most abundant metal found in both the root powders was potassium, with a mean concentration of 6,066.5 mg/kg in *Moringa* root powder and 17,484.33 mg/kg in ginger powder

(Table 6). Potassium is an essential nutrient required for maintaining normal cell function, acid and electrolyte balance, and total body fluid volume in humans (WHO, 2012a). According to WHO, individuals ≥16 years of age should consume at least 3,510 mg of potassium per day (WHO, 2012a). Uptake of this recommended amount of potassium in humans reduces the risk of diseases such as coronary heart disease, stroke and cardiovascular disease and will help regulating blood pressure (WHO, 2012a). The amount of potassium found in ginger and *Moringa* root powders was either comparable or higher than the amount found in potassium-rich unrefined foods such as beans and peas, green vegetables, and fruits. According to WHO, the approximate levels of potassium found in beans and peas, green vegetables and fruits is 13,000 mg/kg, 5,500 mg/kg and 3,000 mg/kg respectively (WHO, 2012a).

Some other metals present in ginger and *Moringa* root powders in high concentrations were calcium, magnesium, sulphur, sodium and phosphorus (Table 6). Each of these metals is deemed important for various biological functions in humans. Magnesium is required for energy production and is a cofactor in about 300 enzyme systems that regulate several biochemical reactions in the human body, including blood glucose control, muscle and nerve function, blood pressure regulation and protein synthesis (ODS, 2020a). The Office of Dietary Supplements (ODS) of U.S. recommends daily uptake of 400-420 mg magnesium in adult males and 310-320 mg magnesium in adult females (ODS, 2020a). This requirement can be easily fulfilled by ginger and *Moringa* root powders, which both had more than 1,000 mg/kg of magnesium present in them (Table 6).

Calcium is the most abundant mineral in the human body that is essential for muscle function, vascular contraction and vasodilation, nerve transmission and hormonal secretion (ODS, 2020b). Calcium supports the structure and function of bones and teeth in human body (ODS, 2020b). According to the Office of Dietary Supplements (ODS) of U.S., Recommended

Dietary Allowances (RDA) for calcium are about 1,000 mg/day in adults (ODS, 2020b). *Moringa* and ginger root powders can help fulfilling this requirement, as they had a mean concentration of 1,810.25 and 966.33 mg/kg calcium present in them (Table 6).

Phosphorus is a component of teeth, bones, DNA, RNA, cell membrane structure and of the key energy source of human body- ATP (ODS, 2020c). It plays an important role in intracellular energy storage, regulating gene transcription and activation of enzymes (ODS, 2020c). Deficiency of phosphorus in human body is quite rare as it is abundant in various foods (Institute of Medicine [US], 1997). In human adults, 700 mg is the average daily intake of phosphorus, as recommended by the Office of Dietary Supplements of US (ODS, 2020c). Ginger and *Moringa* root powders were abundant in phosphorus, with mean concentrations of 6,417.67 and 986 mg/kg respectively (Table 6).

Sulphur is required for synthesis of essential amino acids— cysteine and methionine, and some key proteins (Wong, 2020). It is believed that sulphur protects the human body against osteoarthritis, allergies, muscle soreness, dandruff, rosacea etc. (Wong, 2020). Although there is no Recommended Dietary Allowance (RDA) for sulphur, a daily intake of sulphur-containing amino acids has been recommended. The estimated requirement for methionine (along with cysteine) was determined to be 14 mg/day per kg body weight in adults (Nimni et al., 2007). Both ginger and *Moringa* root powders had high concentration (>1,000 mg/kg) of sulphur present in them and can fulfil the dietary requirements of sulphur in human body (Table 6).

Sodium is an essential element for maintaining pH, fluid balance and cellular homeostasis in human body (Farquhar et al., 2015). The WHO recommends daily intake of <2 grams of sodium in adults (WHO, 2012b). Intake of higher sodium consumption (>2 grams/day) and insufficient potassium (<3.5 grams/day) can contribute to high blood pressure

and can increase the risk of stroke and various other heart diseases in adults (WHO, 2012b). Although one kilogram of ginger and *Moringa* root powders had only about 1.9 and 0.9 grams of sodium respectively (Table 6) but adding common table salt to the diet along with these root powders can fulfil an adult's daily dietary requirement of sodium. The root powders also had high concentration of potassium that can counteract the potential negative effects of high sodium (Morgan et al., 2019).

The most and least abundant metals found in *Moringa* root powder in this research were similar to those found by Morgan et al. (2019) in the dried sample of *Moringa* roots harvested after 7 months of growth (labelled as 'Dry 7 Months'). However, the concentrations of most of these elements were quite low as compared to their levels found by Morgan et al. (2019). This considerable difference in the concentration of elements might have been caused due to variation in pre-harvest conditions of the *Moringa* plants, their post-harvest handling, processing techniques employed and storage conditions (detailed in section 4.2 of this thesis). Shewfelt (1990) found that disparities in all the above-mentioned conditions widely affect the nutrient composition of plants.

The method used to determine concentration of elements present in the *Moringa* roots also varied in the two studies. Morgan et al. (2019) used Inductively coupled plasma-mass spectrometry (ICP-MS), and in this research, inductively coupled plasma- optical emission spectrometry (ICP-OES) was used. ICP-OES measures excited atoms and ions at a wavelength characteristic for specific elements being measured, while ICP-MS measures an atom's mass (Thermo Fisher Scientific, 2022). Therefore, the lower detection limit for ICP-OES is parts per billion but the lower limit for ICP-MS can extend to parts per trillion (Thermo Fisher Scientific, 2022). Thus, ICP-MS is used when the elements to be measured are below or near the lower detection limit of ICP-OES. This might have affected the detected

concentrations of least abundant metals. Although the *Moringa* seeds were ordered from India in both the studies, but the geological region within India from where the seeds were ordered, their growing conditions or any treatments given to the seeds before shipping might have varied in the two studies. This might be another reason behind disparity in the concentration of metals found in the *Moringa* roots.

The concentrations of iron, zinc, lead, and cadmium found in ginger root powder were consistent with their concentrations determined by Wagesho and Chandravanshi (2015). However, magnesium, copper and manganese were present in higher amounts, and calcium, cobalt, chromium, and nickel were found in concentrations lower than those determined by Wagesho and Chandravanshi (2015). This discrepancy in the concentration of metals determined by the two studies can be attributed to differences in mineral concentration of soil media used to grow ginger, soil pH, organic matter content, cation exchange capacity, varieties of ginger grown, and age of the rhizomes used for chemical analysis- as all these factors affect the mineral constitution of a plant (Jung, 2008).

In addition to the essential elements, the concentration of harmful, heavy metals such as lead, mercury, arsenic, cadmium etc. were also determined in ginger and *Moringa* root powders. These metals have been proven to be major health threats as they are harmful for proper functioning of the human body and can interfere with various metabolic processes (Jaishankar et al., 2014). All these metals were present in very low amounts in the root powders, with concentrations less than 10 mg/kg (Table 6). Cadmium was present at <1 mg/kg in the root powders, whereas lead and mercury were present at <2.25 and <3.3 mg/kg respectively. The concentration of arsenic in both the root powders was <6.75 mg/kg (Table 6). The concentration of the heavy metals found in *Moringa* and ginger root powders was consistent

with the concentrations observed by Kaba and Goroya (2019) in roots of *Moringa stenopetala*, and by Goroya et al. (2019) in ginger roots.

The abundance of essential elements such as potassium, calcium, magnesium, sulphur, phosphorus, sodium, and the scarcity of toxic heavy metals like lead, mercury and cadmium in the ginger and *Moringa* root powders justifies their use as a point-of-use water treatment. Occurrence of the essential elements in the root powders in high concentrations would offer additional health benefits and increase the level of nutrients in water.

4.5.3 Total carbon and nitrogen present in Moringa and ginger root powders

Carbon is considered one of the most essential macronutrients as it is required by plants to form carbohydrates, nucleic acid, proteins etc. and is a vital part of plant biomolecules. On an average, the dry weight of a plant cell is 50% carbon. Carbon is rarely limiting as a nutrient, and therefore no specific deficiency symptoms of carbon have been determined in plants. The carbon content of vegetation has been found to be surprisingly constant across a vast variety of tissue species and types. Schlesinger and Bernhardt (1991) found that the carbon content of biomass almost always lies between 45-50% (by oven-dry mass). The results of elemental analysis of this research support this observation as the total carbon concentration detected in *Moringa* and ginger root powders was about 42% (Table 7).

After carbon, nitrogen is one of the important elements present in plants due to its crucial role in chlorophyll production (Muñoz -Huerta et al., 2013). It is also part of several enzymatic proteins that regulate and catalyse plant-growth processes (Sinfield et al., 2010). Furthermore, nitrogen helps in the production of various chemical components that protect plants against parasites and diseases (Muñoz -Huerta et al., 2013). Crop biomass and yield are highly affected by total nitrogen present in the plants or nitrogen fertilization given to the plants

(Tremblay et al., 2011). Total nitrogen is the sum of all different forms of nitrogen, such as nitrate, nitrite, ammonia, reduced and organic nitrogen etc. present in the plants.

Carbon to nitrogen (C:N) ratio of an organic substrate has a direct impact on its composting duration, mineralization and release of nitrogen in the soil that gets available for plant uptake (Brust, 2019). The lower the C:N ratio, more rapidly will the nitrogen be released in the soil. A C:N ratio greater than 35 results in prolonged composting duration and microbial immobilization (Brust, 2019). Root C:N ratios are comparatively higher than the C:N ratios of plant leaves and they do not follow a consistent pattern (Ferlian et al., 2017).

Although differences in climate and conditions such as water availability, soil fertility affect total carbon and nitrogen content, and hence C:N ratio of plants (Luo et al. 2017), Zhang et al. (2014) found that the average carbon and nitrogen content of belowground tissues of most plants growing in Yanqi basin of Northwest China is about 42.8% and 0.7%, respectively. These amounts are similar to the total carbon and nitrogen detected in ginger and *Moringa* root powders through elemental analysis. The average total carbon and nitrogen found in *Moringa* root powder was 42.23% (by oven-dry mass) and 0.25%, respectively, and in ginger powder was 41.90% and 3.7%, respectively (Table 7).

Ginger root powder was found to have about 3.5% more nitrogen (by oven-dry mass) as compared to *Moringa* root powder (Table 7). This could have been due to the fact that being a leguminous plant, ginger is able to fix nitrogen present in soil (Govindan et al., 2009), while *Moringa* is unable to do so (Olson, 2014). This can also explain the higher concentration of total carbon detected in *Moringa* roots than ginger roots, as leguminous plants (including ginger) are able to exchange carbon for nitrogen with their nitrogen-fixing symbionts, and thus, are more responsive to elevated carbon dioxide than non-leguminous plants (Rogers et al., 2009).

The presence of adequate amounts of carbon and nitrogen in ginger and *Moringa* root powder would be beneficial for human health as carbon and nitrogen are the two most essential and vital elements of the human body. Carbon is the second most abundant element that makes up 18.5% of the human body (Chellan and Sadler, 2015). It is a quadrivalent element that allows building of long and complex chains of molecules. This makes it a fundamental and vital atom in organic chemistry. Carbon chains are used for building carbohydrates, proteins and fats, and on breaking, these chains provide energy to the human body (Samir, 2019). On the other hand, nitrogen is a part of essential biomolecules such as proteins, amino acids, nucleobases, DNA and RNA (Chellan and Sadler, 2015).

4.5.4 Concentrations of anions present in Moringa and ginger root powders

Various studies have chemically analysed *Moringa oleifera* and ginger to determine the concentration of essential nutrients such as calcium, magnesium, iron etc. and phytochemicals like glucosinolates, carotenoids, flavonoids, gingerols, shogaols etc. present in their respective plant parts. However, the concentration of various anions found in their plant parts has not been examined. Cation uptake by plants is known to be affected by nutrient content of soil, environmental factors such as temperature, light intensity, moisture availability and genotypic variability of plants (Tang and Rengel, 2003; Anjana and Iqbal, 2007).

Sulphate (SO_4^{2-}) was the most abundant anion present in *Moringa* root powder with a mean concentration of 6,035 mg/kg (Table 8). Although it was present in ginger in higher amounts (9,511 mg/kg), it was the second most abundant anion in ginger root, after phosphate (Table 8). Sulphate is a vital component of extracellular matrix proteins and assists the human body in detoxification of drugs, toxic metals and food additives (Schepker, 2017). It also prevents blood transiting in capillaries from coagulating. Cerebroside sulphate, which is an integral component of the myelin sheaths that surround neuronal axons in the brain, aids in

maintaining optimal neurological health (Schepker, 2017). The human body uses inorganic sulphate in the biosynthesis of essential body compounds (such as dermatan sulphate, keratan sulphate etc.) that cannot be absorbed intact from foods (Institute of Medicine, 2005).

Deficiency of sulphate is believed to cause cardiovascular diseases (Seneff et al., 2015), whereas its overconsumption is known to cause diarrhoea, acidosis, and ulcerative colitis (Institute of Medicine, 2005). Although no-health based guideline value is proposed for sulphate in foods (WHO, 2004), intake of sulphur rich foods such as coconut oil, olive oil, legumes, garlic, onion etc. is recommended to prevent sulphate deficiency (Schepker, 2017). Since ginger and *Moringa* root powders had high concentrations of sulphate (Table 8), they can be helpful in preventing sulphate deficiency.

In human body, almost all phosphorus combines with oxygen to form phosphate (Lewis, 2020). 85% of the body's phosphate is present in bones, while the rest is located inside cells, where it aids the process of energy production (Lewis, 2020). Phosphate is essential for the formation of teeth and bones, and also serves as a building block for cell membranes, adenosine triphosphate (ATP) and nucleic acids (Lewis, 2020). Phosphate concentration in blood can become too high and cause hyperphosphatemia or become too low and cause hypophosphatemia. Although hypophosphatemia is usually asymptomatic, it can cause anorexia, osteomalacia, fatigue and muscle weakness in cases of severe chronic depletion (Imel and Econs, 2012). There is no health-based guideline for daily intake of phosphate, and it is only used as dietary supplement for patients who are unable to get enough phosphorus from their regular diet (Nelson et al., 2017). Ginger and *Moringa* root powders were both abundant in phosphate with 1,029.25 and 12,653.67 mg/kg phosphate present in them respectively (Table 8).

Chloride is the key extracellular anion in humans that is vital for maintenance of acid-base balance, serum electroneutrality, osmotic pressure, fluid homeostasis, renal function and production of hydrochloric acid in gastrointestinal tract (Pfortmueller et al., 2018). Increased levels of chloride in the human body are known to increase blood pressure and hypertension (McCallum et al., 2015). It has also been indicated that increased disease severity in ICU patients is associated with abnormal levels of chloride in their bodies (Pfortmueller et al., 2018). The tolerable upper limit of chloride, which is the maximum level of daily intake that is not known to cause any adverse effects, is 3.6 grams per day in adults (Institute of Medicine, 2005). Both ginger and *Moringa* root powders had chloride amount lower than its tolerable upper limit issued by Institute of Medicine. The average concentration of chloride in ginger and *Moringa* root powders was found to be 737.33 and 454.25 mg/kg (Table 8).

Fluoride is another anion that is considered beneficial for human body as it maintains integrity of teeth and bones. When consumed in optimal amounts, fluoride is known to increase tooth mineralisation, promote dental enamel remineralization, and help reduce dentin hypersensitivity and enamel demineralization (Palmer and Gilbert, 2012). However, acute toxicity of fluoride is known to cause dental fluorosis, bloody vomiting, diarrhoea, hypocalcaemia, hyperkalaemia (Kanduti et al., 2016). In both ginger and *Moringa* root powders, fluoride was present in moderate amounts, with its mean concentration being 182.77 and 178 mg/kg in the root powders respectively (Table 8).

Although nitrate exists widely in water, air, soil and plants, but the chief source of absorbed nitrate in the human body is through food (Ma et al., 2018). Nitric oxide (NO), which is the metabolic product of dietary nitrate, plays an essential role in protecting the gastric mucosa and cardiovascular system (Lundberg et al., 2011). When dietary nitrate is unavailable, the endogenous production of nitrate occurs in mucosa tissues of intestine that performs various

physiological functions similar to NO, such as blood pressure reduction, vessel protective effect and platelet aggregation inhibition (Ma et al., 2018). Nitrate has been found to improve age related hypertension and prevent ischemic heart disease (Ma et al., 2018).

The European Food Safety Authority recommends acceptable daily intake (ADI) of 3.7 mg of nitrate per kg body weight of adults in one day, that is, for a 60 kg human adult, ADI of nitrate would be 222 mg/day (European Food Safety Authority, 2008). The nitrate content in ginger and *Moringa* root powders varied significantly. Where the average nitrate concentration in *Moringa* root powder was only 53 mg/kg, it was 9,326 mg/kg in the ginger powder (Table 8). This means that daily consumption of ginger in very high amounts (>20 g/ day) can be dangerous due to the high concentration of nitrate present in its rhizomes.

The remarkable difference in the amount of nitrate found in the two root powders can be attributed to genotypic variability between the two plant species or variation in their watering frequency or variation in light intensity they received (Anjana and Iqbal, 2007). *Moringa* plants were watered about five times a week, whereas ginger was watered three times a week in summers and only two times a week in winters. Anjana and Iqbal (2007) have shown that plants accumulate less nitrate when they are watered more frequently as frequent irrigation helps in leaching off excess nitrate. *Moringa* plants also received six more hours of supplemental lighting each day than ginger plants. The nitrate content in plants has been found to be inversely correlated to light intensity received by the plants (Anjana and Iqbal, 2007). On decreasing light intensity available to plants, synthesis of soluble carbohydrates and organic acids declines and their role as an osmoticum is taken up by nitrate, which is absorbed and accumulated by plant roots more readily when light is a limiting factor (Anjana and Iqbal, 2007). Thus, future studies should measure changes caused in nitrate content of ginger roots

by providing more water and light to the plants. This might help bringing down the nitrate concentration in ginger roots to a level recommended by European Food Safety Authority.

Bromide and nitrite were the two least abundant anions in ginger and *Moringa* root powders, with their average concentrations being less than 10 mg/kg in both the root powders (Table 8). Since 10 mg/kg was the method detection limit, the exact concentrations of bromide and nitrite in *Moringa* and ginger root powders could not be determined. Nitrite was previously thought to be carcinogenic, and its beneficial functions were ignored. However, most existing research on tumours and nitrite ignored some complicated compounds in target foods, causing contradictory conclusions among researchers (Ma et al., 2018). Considering various protective effects, dietary nitrite seems to play a significant role in physiological functions of the body through its provision of non-enzymatic NO (Ma et al., 2018). Similarly, bromide was once used as a sedative and an anticonvulsant as it is known to cause nausea, abdominal pain, vomiting and even paralysis at large doses, but in smaller amounts, it is considered nutritionally beneficial (WHO, 2009). The World Health Organisation has recommended the upper limit of concentration of daily nitrite uptake to 0.06-0.07 mg/kg (Weitzberg and Lundberg, 2013) and the average daily intake (ADI) of bromide to 0-1 mg/kg body weight (WHO, 2009).

Since the concentration (mg/kg) of anions in ginger and *Moringa* root powders was found to be comparable to their desired amounts in food, it can be concluded that the two root powders can offer additional health benefits and increase the nutrient content of water along with treating water of *E. coli* populations.

4.5.5 Electrical conductivity and pH of Moringa and ginger root powders

Early studies on protoplasmic permeability in plants revealed its association to electrical conductivity (EC). It became evident that the higher the permeability of protoplasm, the more easily will an electric current pass through it. Hence, EC of protoplasm can determine

its permeability to ions (Osterhout, 1918). In early 1900s, EC of plant sap was widely measured to understand problems of plant physiology (Stiles and Jorgensen, 1914) as it was shown that permeability and EC of plant tissues increases when they are injured from mechanical rupture, freezing or from use of chemicals (Dexter et al., 1932). Electrical conductivity in plants has shown to affect water absorption, retention of the absorbed water (Canna©, 2020), water use efficiency and photosynthetic capacity of plants (Xu et al., 1994).

In contemporary greenhouse studies, EC of plant tissues is measured as it is proportional to the total amount of ions and fertilizer salts present in that plant part (Nemali, 2018). Kautkar et al. (2014) found that EC in ginger plants increased with increasing temperature and ionic concentration. Since concentration of all the anions—sulphate, fluoride, chloride, nitrite, bromide, nitrate and phosphate, was found to be higher in ginger roots than in *Moringa* roots (Table 8), EC of ginger was found to be significantly higher than the EC of *Moringa* roots (Table 9).

EC is an important indicator of a plant's health that impacts its nutrient uptake, and thus consequently, affects the plant's growth (Ding et al., 2018). High EC of the ginger roots could be a result of higher concentration of anions (especially nitrate) found in the root powder and/or overfertilization (Ding et al., 2018). As discussed in section 4.5.4, future studies should try bringing down the nitrate content, and consequently EC, of the ginger roots by providing more light and water to the plants to remove excess salt.

Maintaining a stable acid-base status in plant tissues is a fundamental homeostatic process that is necessary for mineral nutrition (Martiniere et al., 2018) and for preserving the metabolic function of macromolecules like proteins (Egginton et al., 1999). Plants can regulate their pH through biochemical or biophysical mechanisms. Biochemical pH regulation involves

chemical transformations that produce or consume H^+, whereas biophysical pH regulation involves transmembrane fluxes of H^+/OH^- (Raven, 1985).

The pH of ginger and *Moringa* root powders was found to be 5.75 and 5.33, respectively (Table 9). These values are consistent to the pH values of ginger roots determined by Williams et al. (2003) and *Moringa* leaves determined by Adetitun et al. (2013). Williams et al. (2003) found that pH of ginger (*Hedychium gardnerianum*) lies between 5.5-6.6, whereas Adetitun et al. (2013) found the pH of aqueous extract of dried *Moringa* leaves to be 5. Since pH of human bodies is partially determined by mineral densities of food they consume, food with pH higher than 4.6 is recommended to reduce chronic diseases and ailments like diabetes, hypertension, vitamin D deficiency, arthritis, low bone density etc. (Levy, 2020). As both the root powders had pH higher than 5, it can be concluded that the *Moringa* and ginger root powders are safe for human consumption. The pH of *Moringa* and ginger root powders was also found to be optimum for their coagulating and water purifying applications as coagulants with slightly acidic pH values (ranging between 5 and 7) are considered favourable for removal of organic matter through charge neutralization mechanism (Naceradska et al., 2019).

4.6 Conclusion

While essential elements such as potassium, calcium, magnesium, sodium etc. were found to be abundant in *Moringa* and ginger root powders, toxic heavy metals like lead, mercury and cadmium were present in meagre amounts in the two root powders (Table 6). Similarly, the root powders were rich in beneficial anions like phosphate, sulphate, chloride etc. (Table 8) and had pH values optimum for human intake (Table 9). The only anion found in concentrations higher than the recommended daily intake in ginger roots was nitrate (Table 8), thus suggesting that ginger should be consumed in smaller quantities (<20 g/day). The high concentration of nitrate in ginger also led to higher EC of ginger roots. Future studies should

try bringing down the nitrate concentration of ginger rhizomes by providing more water and light to the plants. Except nitrate, the concentration of all essential elements and anions in *Moringa* and ginger root powders were found to be optimal. Therefore, it can be concluded that *Moringa* and ginger root powders are safe for human consumption and can offer additional health benefits and increase the nutrient content of water along with treating water of *E. coli* colonies.

Chapter 5: Effect of *Moringa* and ginger root powders on *E. coli* population

5.1 Abstract

Shortage of portable water is a serious problem faced by the world today. Many water treatment methods are available; however, economic, and cultural factors often impair implementation of these methods, particularly in underdeveloped and developing countries. *Moringa* and ginger root powders appear to offer a promising alternative to treat contaminated water. The objectives of this study were to determine the sole and the combined effects of *Moringa* and ginger root powders on ATCC® 8739™ *Escherichia coli* population. The antibacterial efficacy of three replicates of *Moringa* root powder concentrations—800, 1400 and 2000 mg/L, three ginger root powder concentrations—800, 1400 and 2000 mg/L, and three concentrations of the two root powders combined in 1:1 ratio to form 800, 1400 and 2000 mg/L was assessed using IDEXX Colilert-18 Test/Quanti-Tray/2000 Method. The treatments were added to 100 mL buffered milliQ water containing *E. coli* diluted to the factor of $1x10^{-8}$, stirred, and made to sit for an hour before filtering into sample bottles and conducting bacteriological analysis. The mean *E. coli* numbers decreased significantly ($p<0.05$) on adding the root powder concentrations to contaminated water. The *Moringa* roots were found to be more effective in reducing *E. coli* population than ginger roots. The bacterial counts decreased by 84.35%, 88.66% and 86.94% on adding 800, 1400 and 2000 mg/L *Moringa* root powder concentrations, respectively, and by 57.82%, 62.63% and 60.44% on adding the same concentrations of ginger root powder. The combination of the two root powders seemed to have a synergistic effect on *E. coli* as the *E. coli* counts in contaminated water decreased by 90.65%, 93.79% and 94.78% on adding 800, 1400 and 2000 mg/L concentrations of *Moringa* and ginger root powders mixed in 1:1 ratio.

5.2 Introduction

Among all of Earth's natural resources, water is deemed the most important resource as it forms the basic pivotal medium for origin of life (Dwivedi, 2017). Water is critical for survival of all living organisms and has a direct impact on their health (Abiyu et al., 2018). Lack of clean drinking water, also referred to as potable water, is a huge problem faced currently by many sections of population of the world (Varkey, 2018). The contaminants leading to impure water include a wide spectrum of pathogens and chemicals (Ahmed and Ismail, 2018). According to WHO, 80% of the total human diseases are water-borne and claim 5 million deaths globally each year (WHO, 2019). Most of these deaths are attributed to a wide range of microorganisms including *Escherichia coli*, a common microbe found in contaminated water (Cabral, 2010).

Contaminated water has become an environmental issue in urban areas of both developing and developed countries. Although developing countries are marked and characterized by lower industrialization, their wastewater and solid waste management strategies are insufficient, thus facilitating the problem of water contamination (Balthazard-Accou et al., 2019). Moreover, unexpected population growth in developing countries results in water quality degradation and causes an increase in microbial loads in water resources (Balthazard-Accou et al., 2019). Due to these reasons, developing nations in Africa and South Asia are the ones worst affected by the problem of water contamination.

Many cost-effective methods are available to treat contaminated drinking water. These methods are chlorination, boiling, sand filtration and solar disinfection. However, cultural, economic, and social factors often impair implementation of these methods in developing and underdeveloped countries (Morgan et al., 2019). Boiling is the oldest and the most common method of Household Water Treatment (HWT) used by an estimated 1.1 billion

people in 21% of households in middle- and low-income countries (Juran and MacDonald, 2014). It is an effective method known to kill all classes of water-borne pathogens and is even known to treat highly turbid water (Agrawal and Bhalwar, 2009). However, boiling requires consumption of energy that can be costly or not easily available in a lot of regions (Laurent, 2005). It affects the taste of water and does not have any impact on chemical contaminants that might be present in water. In addition to this, there is a high risk for potential microbial recontamination during storage of boiled water (Laurent, 2005).

Chlorination is a simple and inexpensive method that provides a stable residual. Thus, the potential risk of microbial recontamination during storage is lowered (Laurent, 2005). Chlorination is effective against many waterborne pathogens except *Cryptopdoridium parvum*, *Cyclospora cayetanensis*, *Giardia lamblia*, helminth eggs and *Mycobacteria* species (Laurent, 2005). However, the effectiveness of this method is highly dependent on the quality of water as its efficiency is reduced in highly turbid water. There is also some cultural resistance against this method regarding the taste of chlorine (Laurent, 2005).

Solar disinfection and filtration through sand, fabric or paper are other widely used methods for treating water in developing and underdeveloped countries. Solar disinfection involves use of natural UV irradiation to eliminate pathogens present in water. Although this method is inexpensive and does not involve addition of any chemical to water, only small quantities of water can be treated by this method, and it requires good amount of sunshine per day (Laurent, 2005). Sand filtration, on the other hand, is recommended only at community level, and not for individual households because of its large apparatus that needs regular maintenance by trained individuals (Laurent, 2005). Most paper and fabric filters have pore size greater than the diameters of bacteria and viruses, thus reducing its efficiency against these microbes (Agrawal and Bhalwar, 2009).

Water treatment plants in developed and developing countries involve use of chemical coagulants such as ferrous chloride and aluminium sulphate (alum) that can adversely affect human health. For example, aluminium is a recognized neurotoxin that can cause neurodegeneration (Maya et al., 2016) and it has been indicated to be a causative agent of neurological diseases like pre-senile dementia (Abiyu et al., 2018). Therefore, natural coagulants and antimicrobial agents are moving from fringe to mainstream use with majority of people seeking inexpensive water treatment remedies that are free of chemical side effects. These natural antimicrobial agents can also be used in underdeveloped and developing countries where most of the rural population depends on contaminated and turbid water from rivers, streams, and dams (Varkey, 2018).

Moringa (*Moringa oleifera*) and ginger (*Zingiber officinale*) appear to offer a promising alternative to treat contaminated water, since *Moringa* and ginger grow extensively in areas where the problem of contaminated water is quite alarming and various parts of the trees have been reported to possess antibacterial and water purifying properties. *Moringa* seeds were traditionally used in various African countries such as Ethiopia, Uganda, and Sudan for purifying contaminated water and removing its turbidity (Jahn, 1977). Hitherto the seeds have been extensively studied for their water purifying and antibacterial efficacy. Seed extract of *Moringa* has been shown to kill 90-99.99% of bacteria in turbid water (Lea, 2010) and in sedimented sludge (Madsen et al., 1987).

However, utilizing *Moringa* roots in the antimicrobial research would offer added benefits as roots, unlike seeds, can be harvested within the first year of planting the trees (Morgan et al., 2019) and without damaging the tree (Fuglie, 2001). This would reserve the nutrient-rich parts of the shoot such as leaves and seeds to be used as a food source (Morgan et al., 2019). The only research done to evaluate antibacterial efficacy of *Moringa* root powder

used three concentrations (250, 450 and 600 mg/L) and found that the maximum concentration (600 mg/L) reduced *E. coli* colonies by 87% in contaminated pond water (Morgan et al., 2019).

Likewise, ginger extract has been reported to suppress the growth of various Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pyogenes/ pneumoniae, Salmonella typhimurium, Pseudomonas aeruginosa* and *E. coli* (Chrubasik et al., 2005; Gupta and Ravishankar, 2005). However, not much research has been done on ginger's potential to purify contaminated water other than its potential to inhibit growth of a variety of microorganisms that might be present in water.

This chapter focussed on determining whether a further reduction in *E. coli* population can be achieved by increasing the concentrations of *Moringa* and ginger root powders, and whether combining ginger with *Moringa* will have synergistic effect on *E. coli*. Thus, the objectives along with null hypothesis (H_0) and alternative hypothesis (H_a) of this chapter were:

Objective 1: To determine the effect of *Moringa* root powder on *E. coli* population when it was added to the contaminated water at 800, 1,400 and 2,000 mg/L.

> H_0 (1): Increasing concentrations of *Moringa* root powder will have no effect on *E. coli* population.
>
> H_a (1): Increasing concentrations of *Moringa* root powder will result in either decreasing or increasing *E. coli* counts present in contaminated water.

Objective 2: To determine the effect of ginger powder on *E. coli* population when it was added to the contaminated water at 800, 1,400 and 2,000 mg/L.

H_0 (2): Increasing concentrations of ginger root powder will have no effect on *E. coli* population.

H_a (2): Increasing concentrations of ginger root powder will result in either decreasing or increasing *E. coli* counts present in contaminated water.

Objective 3: To determine the combined effects of ginger and *Moringa* root powders on *E. coli* population when both the root powders were mixed in 1:1 ratio to form 800, 1,400 and 2,000 mg.

H_0 (3): Increasing concentrations of amalgamation of *Moringa* and ginger root powders will have no effect on *E. coli* population.

H_a (3): Increasing concentrations of amalgamation of *Moringa* and ginger root powders will result in either decreasing or increasing *E. coli* counts present in contaminated water.

5.3 Methods

5.3.1 Propagating E. coli strain

For preparing 100 mL of LB broth (Luria-Bertani), 2.5 grams of LB broth (Miller) powder was dissolved in 100 mL of deionized water. It was stirred at 700 rpm for 15 minutes using VWR Scientific 220 mini-hot plate stirrer, following which it was autoclaved at 121° C for 15 minutes (P1 liquid cycle). Five millilitres of the freshly prepared LB broth were pipetted into a sterile 16 mL glass test-tube.

For propagating *E. coli*, ATCC® 8739-MINI-PACK™ was used. Extracting *E. coli* from a natural source of contaminated water (pond water shared with domestic animals) was the first preference for this research. However, some practical and logistical problems (detailed in Appendix A) were encountered while collecting and isolating *E. coli* due to which cultured *E. coli* strains were used instead.

A frozen mini-cryovial (Figure 6) containing ATCC® 8739™ *E. coli* was thawed by placing it in room temperature for 20 minutes to melt all the ice crystals present within the vial. Immediately after thawing, the outside surface of the cryovial was wiped with 70% ethanol and its entire contents were emptied into the 16 mL test-tube containing freshly prepared LB broth. The inoculated broth was incubated at 37^0 C for 24 hours.

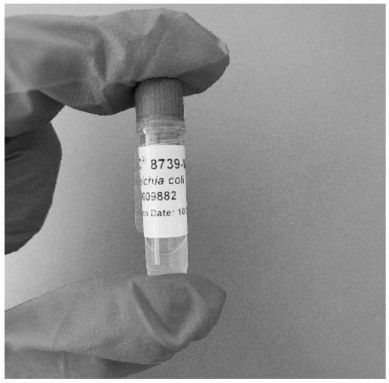

Figure 6. A thawed ATCC® mini-cryovial containing 8739™ *E. coli*. (*Photograph by author, taken on April 12, 2021*).

5.3.2 Preparing streak plates of E. coli

For preparing MUG agar plates, 13.86 grams of powdered MUG agar medium was added to 600 mL of deionized water in a 1000 mL beaker. To mix thoroughly, the solution was stirred on a VWR mini-hot plate stirrer (Model 220) using a magnetic stir bar and was brought to boil. When the powder completely dissolved in the solution, the beaker was covered with aluminium foil and autoclaved at 121^0 C for 15 minutes (P1 liquid cycle).

After autoclaving, the beaker was rested on a counter for 20 minutes until it could be touched by naked hands and before the nutrient media began to solidify. The nutrient agar was gently poured in sterile petri dishes until it covered about half the volume of each petri dish. To prevent condensation, the plates were left on a counter overnight to solidify at room temperature. The next day, MUG agar plates were inverted and stored in a refrigerator at 4^0 C.

A streak plate of *E. coli* was prepared by dipping a sterilized metal loop into inoculated LB broth and streaking it across first quadrant using aseptic technique. The metal loop was sterilized and the bacteria from first quadrant was streaked across the second quadrant. Similarly, the third and the fourth quadrants were streaked through, with the inoculating loop sterilized between each quadrant. The streak plates were then incubated at 37^0 C for 24 hours.

5.3.3 Preparing stock potassium dihydrogen phosphate buffer solution

Recipe of a buffered dilution water mentioned in the book- Standard Methods for the Examination of Water and Wastewater (Baird and Bridgewater, 2017), was followed to prepare a stock phosphate buffer solution. Thirty-four grams of potassium dihydrogen phosphate (KH_2PO_4) were added to 500 mL of deionized water. The solution was stirred using a mini-hot plate stirrer (VWR Scientific 220) and a magnetic stir bar for about 10 minutes until

the potassium salt dissolved completely in the water. The pH of the solution was checked using Orion Star™ A211 Benchtop pH meter and was found to be 4.19. It was adjusted to 7.2 by adding small quantities of 1 mol/L NaOH solution. Distilled water was added to bring the final volume of the buffer solution to one litre. The buffer solution was then autoclaved at 121^0 C for 15 minutes. Once the autoclaved buffer solution reached room temperature, it was stored in a refrigerator at 4^0 C. To prepare buffered dilution water, 1.25 mL of the stock potassium dihydrogen phosphate buffer solution was pipetted to one litre of distilled water.

5.3.4 Serial dilution analysis and enumeration of E. coli

To obtain a countable number of *E. coli* colonies and to ensure that the same standardized *E. coli* numbers are used in each treatment, serial dilution analysis was conducted. Freshly prepared LB broth (5 mL) was placed in a sterile 16 mL test tube and was inoculated with *E. coli*. A single *E. coli* colony was selected from a cultured MUG agar plate and was transferred to the LB broth using a sterile inoculating loop. The inoculated LB broth was then incubated at 37^0 C for 24 hours so that the bacteria entered their stationary growth phase. The approximate time for *E. coli* 8739 to hit the stationary phase has been recorded to be about 6 hours (Wang et al., 2018).

The following day, serial dilutions of 10^{-2}, 10^{-4}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} were prepared from the inoculated broth. The dilution factor of 10^{-2} was prepared by pipetting 1 mL of the inoculated broth into 100 mL of buffered milliQ water. Similarly, 10^{-4} dilution was prepared by pipetting 1 mL of the 10^{-2} dilution into 100 mL of buffered milliQ water. This was repeated to make dilutions of 10^{-6} and 10^{-8}. 10^{-7} and 10^{-9} dilutions were prepared by pipetting 0.1 mL from the 10^{-4} dilution and 1 mL from the 10^{-7} dilution respectively. The dilutions were prepared in sterile 120 mL sample bottles. All the dilutions were rested for 3 hours and 30 minutes before they were analysed to enumerate *E. coli* numbers.

IDEXX Colilert-18 Test/Quanti-Tray/2000 Method (IDEXX® Laboratories, 2013) was used for enumeration of *E. coli* by determining the Most Probable Number (MPN) of *E. coli* present in each of the dilutions. The powdered Colilert-18 reagent was aseptically added to each sample bottle, following which all the bottles were vigorously shaken for two minutes (Figure 7). The dilutions were then poured into sterile Quanti-Trays/2000 (Figure 7), which were then sealed using IDEXX Quanti-Tray™ Sealer (Figure 8). The Quanti-Trays were placed in an incubator at 37^0 C for 24 hours.

Figure 7. Colilert-18 reagent being added to the sample bottles (Left). After mixing Colilert-18 reagent in sample bottles, a sample being poured into Quanti-Tray/2000 (Right). (*Photographs by author, taken on May 13, 2021*).

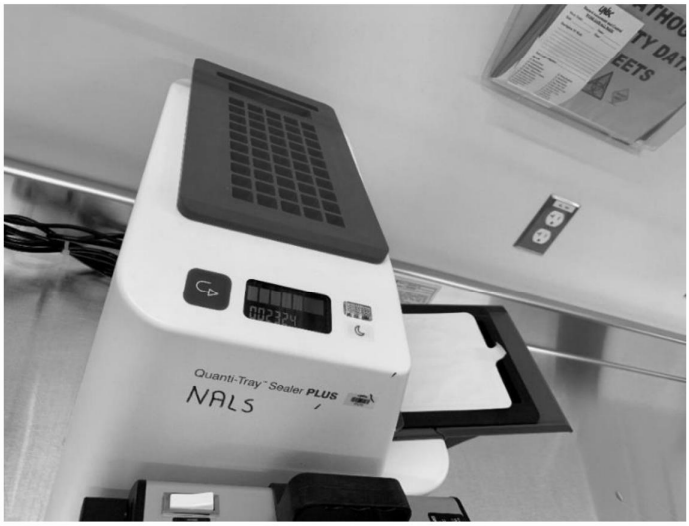

Figure 8. A Quanti-Tray being sealed using IDEXX Quanti-Tray™ Sealer. (*Photograph by author, taken on May 13, 2021*).

After 24 hours, the Quanti-Trays were removed from the incubator and viewed under UV light. The number of wells that fluoresced under the UV light were counted for each Quanti-Tray. The information was compared with IDEXX MPN table to determine MPN of *E. coli* present in each 100 mL of dilution samples. The serial dilution factor of $1x10^{-8}$ was found to be a suitable dilution factor that resulted in countable MPN of *E. coli* per 100 mL of sample.

5.3.5 Evaluating antibacterial effect of Moringa and ginger root powders

Antibacterial efficacy of three concentrations of *Moringa* root powder alone (800, 1400 and 2000 mg/L), three concentrations of ginger root powder alone (800, 1400 and 2000 mg/L), and three concentrations of *Moringa* and ginger root powders combined in 1:1 ratio to form 800, 1400 and 2000 mg/L was assessed. Three replicates of each the above-mentioned concentration were prepared and evaluated for their antibacterial effect on *E. coli*.

In 12, 250 mL glass beakers, 100 mL milliQ water was poured. To each beaker, 125 µL of potassium dihydrogen phosphate stock buffer solution was added and mixed thoroughly

using stirring rods. From each beaker containing 100 mL buffered milliQ water, 10 mL was pipetted out into an empty 250 mL beaker using a glass pipette. All 24 beakers were autoclaved at 121^0 C for 15 minutes.To nine beakers each containing 10 mL milliQ water, 0.08 g, 0.14 g and 0.2 g of *Moringa* root powder was added (each root powder concentration in three beakers). The remaining three beakers with 10 mL of buffered milliQ water and no *Moringa* root powder were used as a control. All the 12 treatments were simultaneously stirred at 500 rpm for 15 minutes on mini hot-plate stirrers (VWR Scientific 220) using magnetic stir bars, after which they were added to the 12 beakers containing 90 mL buffered milliQ water to form 12, 100 mL samples.

Fresh serial dilutions of 10^{-2}, 10^{-4} and 10^{-6} were prepared from an inoculated 5 mL LB broth (that had been incubated for 24 hours after its inoculation). One millilitre from the dilution factor of $1x10^{-6}$ was pipetted into each 100 mL sample to give rise to standardized bacterial concentration of a dilution factor $1x10^{-8}$ per 100 mL sample. All the 12 samples were stirred at 500 rpm for 10 minutes on VWR mini hot-plate stirrer to thoroughly mix the bacterial concentrations with the *Moringa* root powder. After this, the samples were left to sit for one hour before performing bacterial enumeration procedures.

After one hour, the samples were filtered into sterile 120 mL sample bottles using Whatman 150 mm, grade 2 filter paper (catalogue number: 1002-147). Total time taken by the samples to filter through the filter paper varied from about 45 minutes to two hours, depending on the concentration of root powders added to the samples. While the controls (consisting of only milliQ water and no root powder concentration) took about 45 minutes to filter through the Whatman grade 2 filter paper, samples with 2000 mg/L *Moringa* root powder took about two hours to complete the filtration process. On completing 3 hours and 30 minutes after making the standardized bacterial concentrations, the sample bottles were capped,

appropriately labelled, and analysed using IDEXX Colilert-18 Test/Quanti-Tray/2000 Method (IDEXX® Laboratories, 2013). After 24 hours, the Quanti-trays were removed from the incubator and *E. coli* present in each sample were enumerated using MPN reference table.

Similarly, the antibacterial effect of ginger root powder concentrations (three replicates of 0, 800, 1400 and 2000 mg/L each) and the combined effect of the two root powders mixed (three replicates of 0, 800, 1400 and 2000 mg/L each) on standardized *E. coli* numbers was evaluated. To study the synergistic antibacterial effect of the two root powders, *Moringa* and ginger root powders were mixed in 1:1 ratio. For instance, 400 mg each of *Moringa* and ginger root powder was mixed to evaluate the effect of their combined concentration (800 mg/L) on *E. coli* population.

5.3.6 Statistical analysis

Statistical analysis of the data was performed by R Studio software. Mean and standard deviation, which were used to present results graphically, were calculated. A one-way Analysis of Variance (ANOVA) and a Tukey post hoc comparison test was performed to ascertain the significance of the effect of *Moringa* and ginger root powders on *E. coli* population. Significance of all tests was determined at a p-value of 0.05.

5.4 Results

5.4.1 Propagating E. coli strain

ATCC® 8739™ *E. coli* was grown on MUG agar plates using streak plate technique. The *E. coli* colonies on MUG agar plate appeared off-white and creamy in colour and had a shiny texture. The colonies were circular, smooth, opaque and were slightly raised (Figure 9). The *E. coli* colonies growing on MUG agar plate fluoresced when viewed under UV light, thus confirming that the bacterial growth on MUG agar plates consisted of pure *E. coli* colonies.

Figure 9. A streak MUG agar plate with *E. coli* colonies. (*Photograph by author, taken on May 6, 2021*).

Figure 10. Comparison of sterile LB broth with inoculated LB broth. *(Photograph by author, taken on May 12, 2021*).

When a single *E. coli* colony was selected from MUG agar plate and transferred to sterile LB broth, the inoculated LB broth was incubated for 24 hours before using the broth for subsequent analysis. After 24 hours, the inoculated LB broth appeared cloudy with sediments settled at the bottom of the test-tube (Figure 10), whereas sterile LB broth was clearer and transparent.

5.4.2 Serial dilution analysis

The dilution factor of $1x10^{-8}$ was found to be suitable and resulted in a countable 1299.7 MPN of *E. coli* per 100 mL of sample. The Quanti-trays with the samples of dilution factors— $1x10^{-2}$, $1x10^{-4}$, $1x10^{-6}$ and $1x10^{-7}$ were all saturated and showed *E. coli* counts (>2419.6 MPN of *E. coli*/100 mL of dilution sample) higher than the bacterial detection limit of IDEXX Colilert-18. The dilution factor of $1x10^{-9}$ resulted in 11.8 MPN *E. coli*/100 mL of sample. Hence, the $1x10^{-8}$ dilution factor was found to be adequate and was used in all antibacterial analyses.

5.4.3 Antibacterial effect of Moringa and ginger root powders

As described in part 5.3.5 of this thesis, the antibacterial effect of three *Moringa* root powder concentrations— 800, 1400 and 2000 mg/L, was determined on standardized *E. coli* numbers. Three replicates of each *Moringa* root powder concentration along with three replicates of control (0 mg/L of *Moringa* in 100 mL of milliQ water) were analysed using IDEXX Colilert-18/Quanti-Tray/2000 Method.

5.4.3.1 Moringa root powder treatments

The mean *E. coli* counts decreased from 677.33 MPN to 104.13, 74.6 and 87.53 MPN on adding 800, 1400 and 2000 mg/L *Moringa* root powder to the contaminated water, respectively (Figure 11), thus rejecting the null hypothesis [H_0 (1)].

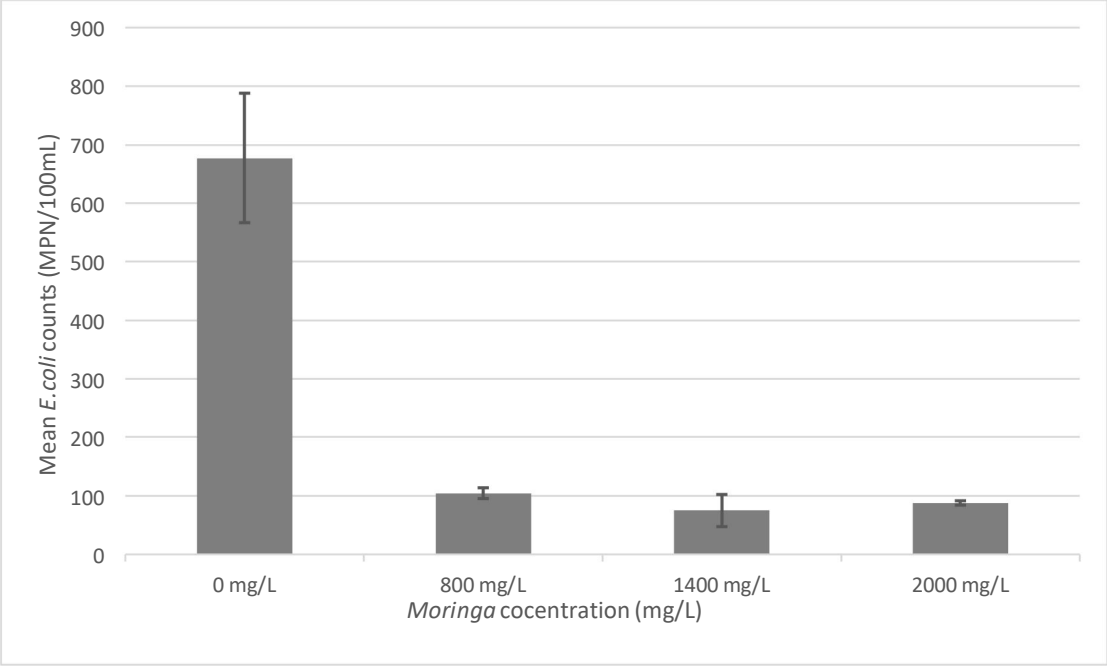

Figure 11. Mean *E. coli* counts found in different *Moringa* root powder treatments, with standard deviation as error bars.

A one-way ANOVA revealed that a significant decrease ($F(3) = 136.38$, $p = 3.30e-07$) in *E. coli* population was observed on adding *Moringa* root powder concentrations to contaminated water (Table 10). A post hoc Tukey test showed that *E. coli* counts in each of the *Moringa* concentration— 800, 1400 and 2000 mg/L, varied significantly ($p<0.05$) from the bacterial counts in control (Table 11). However, a significant difference in *E. coli* numbers was not observed within 800 and 1400 mg/L ($p = 0.84$), 800 and 2000 mg/L ($p = 0.97$), and 1400 and 2000 mg/L *Moringa* treatments ($p = 0.98$).

Table 10. One-way analysis of variance for different *Moringa* root powder treatments— 800, 1400 and 2000 mg/L, on mean *E. coli* counts. A significant difference is considered at p-value <0.05.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	p-value
Moringa treatments	3	780768.6	260256.2	136.38	3.30e-07
Residuals	8	15265.94	1908.2425	-	-

Table 11. A Tukey's test showing differences in means, confidence levels (upper and lower limit) and adjusted p-values for all possible pairs of different concentrations of *Moringa* root powder at 95% family-wise confidence level.

Treatment groups	Difference in means	Lower limit	Upper limit	Adjusted p-value*
800 and 0 mg/L	-573.20000	-687.4195	-458.98051	0.0000011
1400 and 0 mg/L	-602.73333	-716.9528	-488.51384	0.0000007
2000 and 0 mg/L	-589.80000	-704.0195	-475.58051	0.0000009
1400 and 800 mg/L	-29.53333	-143.7528	84.68616	0.8399143
2000 and 800 mg/L	-16.60000	-130.8195	97.61949	0.9645943
2000 and 1400 mg/L	12.93333	-101.2862	127.15282	0.9825329

* Significant at p-value <0.05

5.4.3.2 Ginger root powder treatments

The antibacterial effect of three ginger root powder concentrations— 800, 1400 and 2000 mg/L, and a control with 100 mL of milliQ water and no ginger powder (three replicates of each treatment) was determined using IDEXX Colilert-18. The mean *E. coli* numbers in control were found to be 1085.07 MPN, but the bacterial counts decreased significantly (F (3) =12.75, p= 0.002) in treatments with ginger root powder (Table 12), thus rejecting the null hypothesis [H_0 (2)]. The mean *E. coli* counts in treatments with 800, 1400 and 2000 mg/L ginger root powder concentrations were found to be 433.4, 389.1 and 427.87 MPN respectively (Figure 12).

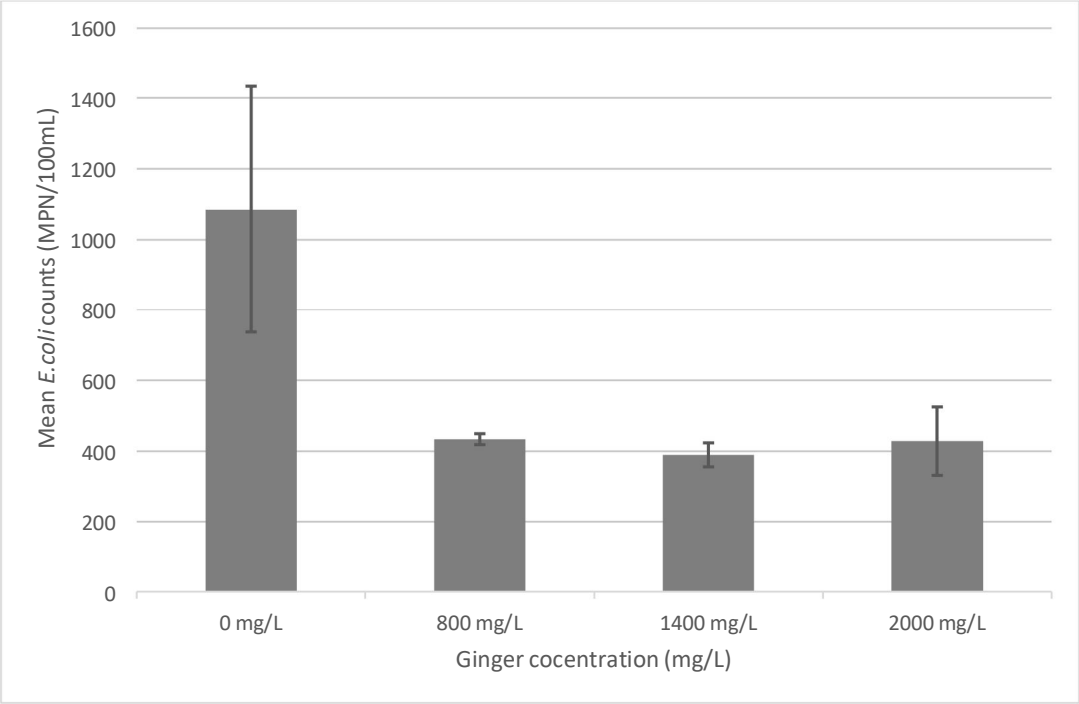

Figure 12. Mean *E. coli* counts found in different ginger root powder treatments, with standard deviation as error bars.

Table 12. One-way analysis of variance for different ginger root powder treatments— 800, 1400 and 2000 mg/L, on mean *E. coli* counts. A significant difference is considered at p-value <0.05.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	p-value
Ginger treatments	3	1008335.13	336111.71	12.75	0.002
Residuals	8	210849.39	26356.17	-	-

A post hoc Tukey test revealed that the *E. coli* numbers in the control were significantly higher than in the treatments with 800 mg/L (p= 0.005), 1400 mg/L (p= 0.003) and 2000 mg/L ginger powder (p= 0.005). However, a statistically significant difference in *E. coli* numbers was not observed within treatments with ginger root powder concentrations as adjusted p-value was found to be higher than 0.05 in all these cases (Table 13).

Table 13. A Tukey's test showing differences in means, confidence levels (upper and lower limit) and adjusted p-values for all possible pairs of different concentrations of ginger root powder at 95% family-wise confidence level.

Treatment groups	Difference in means	Lower limit	Upper limit	Adjusted p-value*
800 and 0 mg/L	-651.666667	-1076.1539	-227.1794	0.0051241
1400 and 0 mg/L	-695.966667	-1120.4539	-271.4794	0.0034215
2000 and 0 mg/L	-657.200000	-1081.6872	-232.7128	0.0048683
1400 and 800 mg/L	-44.300000	-468.7872	380.1872	0.9861884
2000 and 800 mg/L	-5.533333	-430.0206	418.9539	0.9999715
2000 and 1400 mg/L	38.766667	-385.7206	463.2539	0.9906214

*Significant at p-value <0.05

5.4.3.2 Moringa and ginger root powder treatments

The mean *E. coli* numbers decreased from 1901.87 MPN (in control) to 169.87, 118.1 and 96.17 MPN on adding 800, 1400 and 2000 mg/L *Moringa* and ginger root powders combined in 1:1 ratio to contaminated water respectively (Figure 13), thus rejecting the null hypothesis [H_0 (3)].

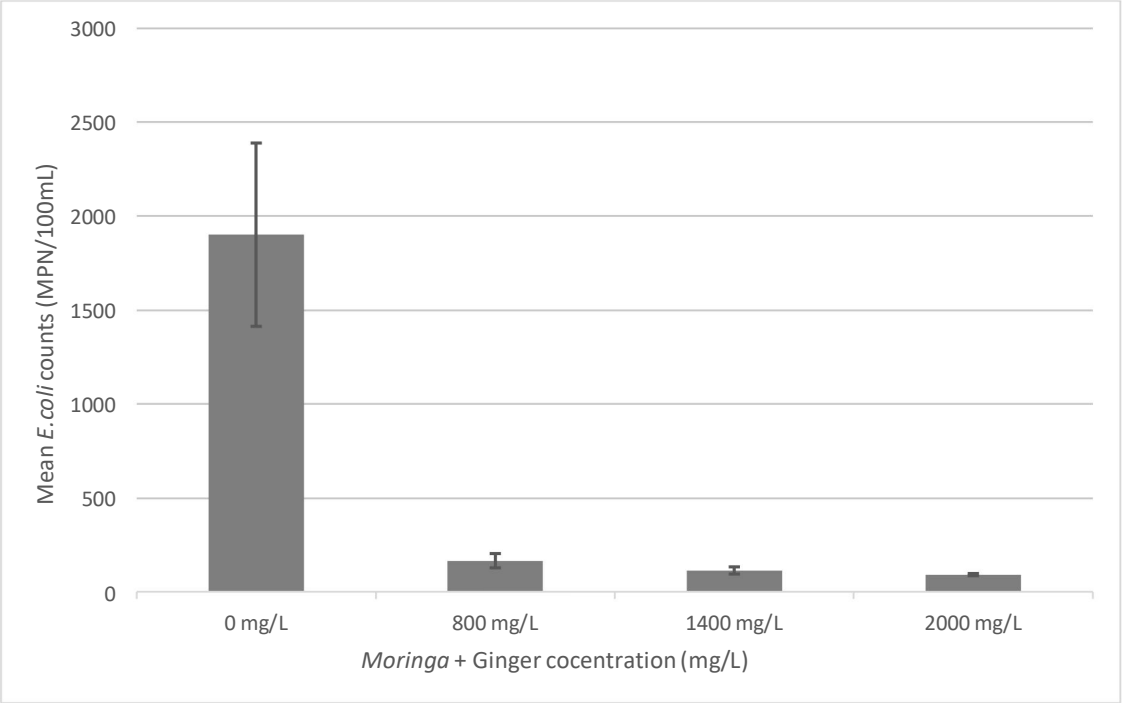

Figure 13. Mean *E. coli* counts detected in treatments with *Moringa* and ginger root powder concentrations, with standard deviation as error bars.

The *E. coli* counts decreased significantly (F (3) =44.46, p= 2.47e-05) on adding the combination of both *Moringa* and ginger root powder to contaminated water (Table 14). Table 15 shows results of the Tukey's test, according to which *E. coli* counts in each of the treatments with the root powders varied significantly ($p<0.05$) from the bacterial counts in control, but a significant difference in *E. coli* numbers was not observed within treatments with 800 and 1400

mg/L (p= 0.99), 800 and 2000 mg/L (p= 0.98), and 1400 and 2000 mg/L *Moringa* and ginger root powders (p= 0.99).

Table 14. One-way analysis of variance for different *Moringa* and ginger root powder treatments— 800, 1400 and 2000 mg/L, on mean *E. coli* counts. A significant difference is considered at p-value <0.05.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value	p-value
Moringa+ ginger treatments	3	7088094.42	2362698.14	44.46	2.47e-05
Residuals	8	425112.47	53139.05	-	-

Table 15. A Tukey's test showing differences in means, confidence levels (upper and lower limit) and adjusted p-values for all possible pairs of different concentrations of *Moringa* and ginger root powder at 95% family-wise confidence level.

Treatment groups	Difference in means	Lower limit	Upper limit	Adjusted p-value*
800 and 0 mg/L	-1732.00000	-2334.7405	-1129.2595	0.0000731
1400 and 0 mg/L	-1783.76667	-2386.5072	-1181.0262	0.0000588
2000 and 0 mg/L	-1805.70000	-2408.4405	-1202.9595	0.0000538
1400 and 800 mg/L	-51.76667	-654.5072	550.9738	0.9921605
2000 and 800 mg/L	-73.70000	-676.4405	529.0405	0.9782437
2000 and 1400 mg/L	-21.93333	-624.6738	580.8072	0.9993845

*Significant at p-value <0.05

The mean percentage decreases observed in *E. coli* counts on adding different concentrations of *Moringa* root powder alone, ginger root powder alone and the combination of the two root powders were calculated and compared. In *Moringa* root powder concentrations, maximum percentage decrease in *E. coli* numbers was observed in 1400 mg/L— 88.66%, as compared to 800 and 2000 mg/L concentrations that caused 84.35% and 86.94% reduction in *E. coli* respectively (Figure 14). Although the *E. coli* numbers present in

1400 mg/L treatment were not significantly different from the bacterial counts in 800 and 2000 mg/L treatments (Table 11), 1400 mg/L was considered the most effective concentration of *Moringa* root powder as it caused higher percentage decrease in *E. coli* population than achieved by 800 and 2000 mg/L concentrations (Figure 14). Like in the case of *Moringa* root powder, 1400 mg/L ginger root powder concentration was found to be the most effective at reducing *E. coli* numbers. Where 1400 mg/L ginger root powder concentration reduced *E. coli* numbers by 62.63%, treatments with 800 and 2000 mg/L concentrations reduced bacterial counts by just 57.82% and 60.44% respectively (Figure 14).

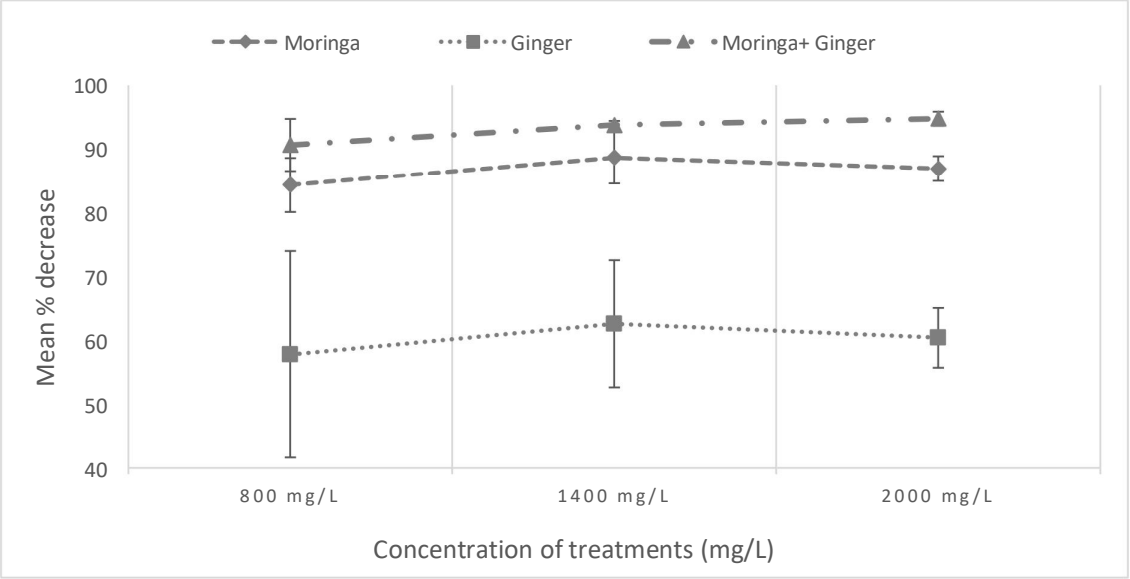

Figure 14. Mean percentage decrease in *E. coli* numbers observed in different concentrations of *Moringa* root powder alone, ginger root powder alone and the combination of the two root powders, with standard deviation as error bars.

Moringa and ginger root powders combined in 1:1 ratio, reduced bacterial counts in contaminated water more efficiently than either of the two root powders alone. Out of the three concentrations of combined *Moringa* and ginger root powders, maximum percentage decrease in *E. coli* numbers was caused by 2000 mg/L— 94.78%, as compared to 800 and 1400 mg/L concentrations that reduced *E. coli* counts by 90.65% and 93.79% respectively (Figure 14).

5.5 Discussion

Moringa root powder was found to be more effective in reducing *E. coli* numbers in contaminated water than ginger root powder. The *Moringa* root powder concentration with highest antibacterial efficacy against *E. coli* was found to be 1400 mg/L. It reduced the *E. coli* counts in contaminated water by 88.66%, whereas 800 and 2000 mg/L *Moringa* root powder concentrations reduced the bacterial counts by 84.35% and 86.94% respectively (Figure 14). These values were similar to the literature values for percentage reduction in bacteria caused by seed paste or seed extract of *Moringa oleifera*. Madsen et al. (1987) reported a 90% reduction of bacterial load in water treated with *Moringa* seed paste and Oluduro and Aberiye (2007) reported that *Moringa* seed extract reduced about 88% of the total bacteria in surface water.

The percentage decrease observed in *E. coli* was similar to the values reported by Morgan et al. (2019), who determined that *Moringa* root powder had a maximum efficiency of 87% reduction in *E. coli* when added to contaminated water at 600 mg/L. A similar percentage decrease (88.66%) in *E. coli* numbers was achieved by adding more than double the amount of *Moringa* root powder used by Morgan et al. (2019). This differential antibacterial efficacy of the *Moringa* root powder concentrations in the two studies might have arisen due to variation in pre- and postharvest handling of the *Moringa* plants used in the two studies and due to the differences in concentrations of various chemical components reported in their root powders. The *E. coli* strain used in the antibacterial tests might be different in the two studies as well. While ATCC® 8739™ strain of *E. coli* was used in this research, Morgan et al. (2019) extracted an unspecified strain of *E. coli* from an agricultural pond. This might be another reason behind the discrepancy in antibacterial effect displayed by *Moringa* root powder concentrations in the two studies.

Comparing the antibacterial efficacy of *Moringa* root powder found in this study with the results attained by Morgan et al. (2019), it appears that very little incremental death in *E. coli* population is attained by increasing the *Moringa* root powder concentrations past 600 mg/L. Morgan et al. (2019) found significant ($F(1,6) = 74.27$, $p = 0.0001$) reduction of 87% in *E. coli* by increasing *Moringa* root concentration from 0 to 600 mg/L. However, past 600 mg/L, not much further reduction in *E. coli* was achieved, as 800, 1400 and 2000 mg/L *Moringa* root powder concentrations were able to achieve only 84.35%, 88.66% and 86.94% percentage decrease in *E. coli* numbers (Figure 14). A significant difference in *E. coli* numbers was not observed between 800 and 1400 mg/L ($p = 0.84$), 800 and 2000 mg/L ($p = 0.97$), and 1400 and 2000 mg/L ($p = 0.98$) *Moringa* treatments (Table 11). With little increment observed in death of *E. coli* after adding *Moringa* root powder concentrations past 600 mg/L, and as the bacterial population increased after increasing the root powder concentration from 1400 mg/L to 2000 mg/L, it seems like 1400 mg/L is the most effective and the highest *Moringa* root powder concentration that can reduce *E. coli* colonies in contaminated water.

The bioactive component of *Moringa* roots responsible for killing *E. coli* in contaminated water was not ascertained in this research. Eilert et al. (1981) identified 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate as the active antibacterial component present in seeds of *M. oleifera* and *Moringa stenopetala*. Benzyl isothiocyanate is known to target cellular proteins of bacteria, resulting in disruption of major metabolic processes and eventually leading to cell death (Dufour et al., 2013). Ganatra et al. (2012) found 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate and pterygospermin to be the chief chemical constituents accountable for antibacterial activity of *M. oleifera* Lam. Out of these three constituents, roots of *M. oleifera* are known to contain 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate (Ganatra et al., 2012). Thus, the bioactive component of *Moringa* roots responsible for killing *E. coli* might be 4-(α-L-

rhamnopyranosyloxy) benzyl glucosinolate. However, further research needs to be done to confirm this proposition and to determine if its mode of action is similar to benzyl isothiocyanate or *Moringa oleifera* cationic protein (described in section 2.1.6 of the thesis).

Ginger root powder was found to be less effective at reducing *E. coli* counts in the contaminated water than *Moringa* root powder (Figure 14). However, like *Moringa* root powder, 1400 mg/L concentration of ginger root powder caused higher percentage decrease in *E. coli* numbers than 800 and 2000 mg/L ginger concentrations, thus making 1400 mg/L the highest and the most effective ginger root powder concentration capable of reducing *E. coli* colonies in contaminated water. Ginger root powder reduced *E. coli* numbers by 57.82%, 62.63% and 60.44% when added to contaminated water at 800, 1400 and 2000 mg/L concentration respectively (Figure 14). This is in accordance with various previous studies that determined antibacterial effect of ginger extracts on numerous bacteria (Malu et al., 2009; Karuppiah and Rajaram, 2012; Islam et al., 2014; Yadufashije et al., 2020; Wang et al., 2020). However, a direct comparison of percentage decrease in *E. coli* caused by ginger root powder cannot be drawn with the previous studies as all the above-mentioned studies investigated inhibition zone caused in bacterial growth by ginger extracts or powder. None of the previous studies enumerated *E. coli* numbers before and after applying ginger powder concentrations to the contaminated water.

This research did not identify the bioactive compound of ginger rhizomes responsible for killing the *E. coli* population. Mao et al. (2019) found phenolic compounds such as gingerols, shogaols and paradols to be the chief bioactive components present in ginger. Zingerone and 6-shogaol (both formed from gingerol), zingiberene, 6-gingerol, α-curcumene (present in ginger's essential oil), 10-gingerol and 12-gingerol are some of the bioactive components responsible for antibacterial efficacy of ginger (Mao et al., 2019; Wang et al.,

2020). While ginger essential oil and its components are known to inhibit gene expression of bacterial energy metabolism, cell membrane related proteins, and DNA metabolism (Wang et al., 2020), gingerol and its components are believed to affect membrane integrity and inhibit biofilm formation in bacterial cells (Mao et al., 2019). Further research needs to be done to identify the specific bioactive compound (out of the above-mentioned compounds) present in ginger rhizomes that kills *E. coli* colonies in contaminated water and determine its mode of action.

In both the cases of *Moringa* and ginger, the antibacterial effect of different concentrations of the root powders on *E. coli* showed an inverted bell-shaped trend (Figure 11, 12). That is, the *E. coli* numbers decreased on adding 800 mg/L of the root powders to contaminated water. A further decrease in *E. coli* population was noticed when the concentration of the root powders was increased from 800 to 1400 mg/L. However, following 1400 mg/L, no further increment in the percentage reduction of *E. coli* was observed and thus, the *E. coli* counts enumerated in treatments with 2000 mg/L concentration of the two root powders was higher than the bacterial numbers found in treatments with 1400 mg/L (Figure 11,12). Thus, the antibacterial properties of *Moringa* and ginger root powders appear to be dose dependent. This can either be because at higher concentrations, the nutritional potency of root powders surpasses their antibacterial efficacy, or at higher concentrations, the phytochemical constituents of the root powders alter the water conditions in a way that favours *E. coli* growth. Further research needs to be done to understand why after a certain concentration, the antibacterial effect of *Moringa* and ginger root powders on *E. coli* population begins to drop.

Moringa and ginger root powders appeared to have a synergistic effect on *E. coli*. The combination of the two root powders mixed in 1:1 ratio showed a higher percentage reduction in *E. coli* numbers than shown by either of the root powders alone. On adding 800,

1400 and 2000 mg/L concentration of the two root powders mixed in 1:1 ratio, the *E. coli* counts in contaminated water decreased by 90.65%, 93.79% and 94.78% respectively (Figure 14). According to Stefanovic (2017), bioactive components of plant extracts have ability to modify or block the resistance mechanism of a bacteria, thus making the bacteria sensitive to another plant extract used along with it or an antibiotic. Thus, the antibiotic (or another plant extract) can produce similar antibacterial results at lower concentration when added along with a plant extract (Stefanovic, 2017). Future studies should try to examine the mode of actions of *Moringa* and ginger root powders on *E. coli*, and the combined mode of action of their respective bioactive compounds on *E. coli*. This would explain the rationale behind synergistic effect of amalgamation of the two root powders on *E. coli*.

Standard deviation, displayed as error bars in the Figures 11, 12 and 13, varies greatly among different concentrations of *Moringa* and ginger root powder. Although standardized *E. coli* numbers were used in each replicate of a treatment (obtained by adding the same dilution factor—$1x10^{-8}$), the initial population of *E. coli* pipetted into each sample could have varied to some extent, thus resulting in greater standard deviation among some treatments than others. Another reason behind disparity in *E. coli* numbers reported in treatment replicates might be the discrepancy in weights of root powders used. The sartorius weighing balance used to weigh root powders displayed values up to two decimal places. For instance, 0.08 gram of root powder was added to 100 mL of sample for preparing 800 mg/L treatment concentration. So, the root powder used in different replicates of 800 mg/L concentration could have varied from 0.081 gram to 0.089 grams, or from 81 mg to 89 mg. Future studies should use a weighing balance capable of weighing root powders up to three decimal places to ensure less discrepancy among replicates of a single root powder concentration.

Chapter 6: Conclusion and Recommendations

6.1 Conclusion

This study aimed to document the methods and techniques used to grow *Moringa oleifera* and *Zingiber officinale* (ginger) plants in a greenhouse, analyse their growth, harvest, and process their roots, study inorganic elemental composition of their root powders and determine their antibacterial efficacy against *E. coli*. The *Moringa* seeds were obtained from India and were planted in I.K. Barber Enhanced Forestry Lab (EFL) greenhouse at UNBC for seven months, whereas the ginger rhizomes were purchased from a supermarket and were grown in the greenhouse for about three years. The *Moringa* and ginger roots were harvested, washed, peeled, freeze-dried, and ground to a fine powder using an electronic grinder.

Inductively coupled plasma- optical emission spectrometry (ICP-OES) was used to measure concentrations of 27 metals in the root powders of *Moringa* and ginger. The most abundant metal found in both the root powders was potassium, while calcium, magnesium, sulphur, sodium, and phosphorus were present in high concentrations (each >1,000 mg/kg). Harmful and heavy metals such as lead, mercury, arsenic and cadmium were present in the root powders in very low concentrations— each less than 10 mg/kg. Concentrations of anions such as sulphate, phosphate, chloride, and nitrate were found to be significantly higher in ginger than in *Moringa* root powder. Both the root powders were found to be slightly acidic with pH values lying between 5 and 6.

The antibacterial efficacy of the root powders was determined using IDEXX Colilert-18 Test/Quanti-Tray/2000 Method. The mean *E. coli* numbers decreased significantly ($p<0.05$) on adding different concentrations of *Moringa* and ginger root powder to contaminated water. The *Moringa* roots were more effectual in reducing *E. coli* population than ginger roots. For both *Moringa* and ginger root powder, 1400 mg/L concentration was

determined to be the most effective concentration as it reduced *E. coli* in contaminated water by 88.66% and 62.63% respectively. The combination of the two root powders was found to have a synergistic effect on *E. coli* as *Moringa* and ginger root powder combined in 1:1 ratio reduced bacterial counts more proficiently than either of the two root powders alone. The *E. coli* counts in contaminated water decreased by 90.65%, 93.79% and 94.78% on adding 800, 1400 and 2000 mg/L concentrations of mixed *Moringa* and ginger root powder.

This research sought to explore the potential use of *Moringa* and ginger roots in treating contaminated water, which is a very common problem in underdeveloped and developing countries. The abundance of essential elements and the scarcity of toxic, heavy metals in the two root powders validates their use as a point-of-use water treatment. The presence of essential elements in the root powders in high concentrations will offer additional health benefits along with treating water of *E. coli* colonies.

6.2 Recommendations

This research determined 1400 mg/L as the most effective concentration for both *Moringa* and ginger root powders in reducing *E. coli* counts. However, the most effective concentration of the combination of *Moringa* and ginger root powders (mixed in 1:1 ratio) could not be determined as the *E. coli* numbers still seemed to be decreasing by increasing the concentration of amalgamation of the root powders following 2000 mg/L (the highest concentration of the root powders tested). Thus, further studies need to be conducted to determine a specific concentration of amalgamation of *Moringa* and ginger root powders which, when added to contaminated water, reduces the *E. coli* counts to the lowest number possible.

Future studies should conduct a direct comparison between antibacterial and chemical properties of *Moringa* and ginger root powders by growing *Moringa* and ginger plants

in a greenhouse under similar conditions and for equal length of time. The bioactive compounds within *Moringa* and ginger roots responsible for killing *E. coli* colonies in contaminated water and their respective mode of actions should also be investigated in the future.

Since *E. coli* population in contaminated water did not reach WHO's required standard of <1 CFU/100 mL (WHO and UNICEF 2017), further research needs to be done to develop *Moringa* and ginger roots as a water treatment method that can produce more viable results. The two root powders could be combined with crushed *Moringa* seeds, leaves or with other plant extracts known to possess antibacterial properties. A comparison could also be drawn between antibacterial efficacy of the *Moringa* and ginger roots harvested from plants belonging to different age groups. Lastly, water treated with *Moringa* and ginger root powders should be assessed for physical water quality parameters such as turbidity, pH etc., palatability and social acceptance in order to successfully develop the two root powders as a point-of-use water treatment method in underdeveloped and developing countries.

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Appendix A. Extracting *E. coli* used in bacteriological analysis (Trial method)

Since the primary aim of this research was to determine the effect of *Moringa* and ginger root powder on *E. coli* population, extracting *E. coli* from a natural source of water was the first preference. The *E. coli* strains extracted from a natural water source would be genetically closer and behave similar to the *E. coli* strains found in open water sources in underdeveloped and developing countries, than the K-12 *E. coli* or other cultured strains of *E. coli* belonging to Risk Group 1. Generally, open water sources are used to draw drinking water in countries where *Moringa* and ginger grow naturally and where the problem of contaminated water is quite alarming.

Water was collected from a pond located in an agricultural farm close to UNBC and in the north of Prince George, on November 18, 2020. This pond was selected due to the natural presence of *E. coli* in its water, as determined by Morgan et al. (2019). In addition to this, the bacteriological contamination and the physical properties of the pond water represented the real-world water quality conditions in open water sources (Morgan et al. 2019). Five litres of pond water were collected from mouth of a pipe feeding the frozen pond (Figure 15) in a 20L sterilized plastic container (Reliance Rectangular Aqua Pak water container). The container was rinsed thrice with the pond water before collecting the water sample according to CCME standards (CCME 2011).

The environmental parameters—temperature, pH, turbidity, and electrical conductivity, of the pond water were measured on the same day to understand the baseline characteristics of the water used for testing. Three readings of each environmental parameter were taken and a mean value for each of them was calculated. The mean temperature, pH, electrical conductivity and IR ratio (turbidity) of the pond water was calculated to be 0.23^0 C, 6.85, 71.28 µS/cm and 9.89 respectively.

Figure 15. The opening (mouth) of pipe from where water sample was collected, and environmental parameters of the pond water were measured. (*Photograph by author, taken on November 19, 2020*).

The pond water sample was brought back to UNBC. The plastic container was shaken and swirled for a few minutes to thoroughly mix the water sample before removing 400 mL of the sample. This 400 mL water sample was used to extract and isolate *E. coli* using serial dilutions and membrane filtration. The water sample was diluted by a factor of 2, 10 and 100 using milliQ water. These dilutions along with an undiluted pond water sample were run through 0.45 µm membrane filters. Using sterile forceps, the membrane filters were transferred to petri-dishes with poured m-ColiBlue® broth ampules. m-coli blue media was used for selecting *E. coli* and coliform colonies growing on the filter. The petri-dishes were incubated for 24 hours at 35^0 C.

After 24 hours, a single *E. coli* colony (blue in colour) and a few coliform colonies (red in colour) were observed on filter with water sample diluted by a factor of 2 (Figure 16).

Water samples diluted by a factor of 10 and 100 showed no coliform or *E. coli* growth, whereas the undiluted sample depicted growth of a few coliform colonies, but not a single *E. coli* colony. Extraction of just one *E. coli* colony from four different dilutions of pond water could be a result of low temperature of pond water used for isolating *E. coli*. Since the pond water sample was collected in the middle of November 2020, most parts of the pond were frozen. Due to this, the water had to be collected from mouth of a pipe feeding the pond as it was the only spot with flowing water.

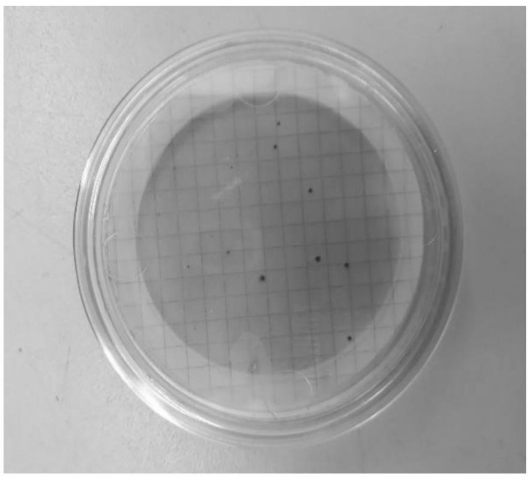


Figure 16. An *E. coli* colony (blue in colour) and numerous coliform colonies (red in colour) observed on a membrane filter with m-ColiBlue media. (*Photograph by author, taken on November 20, 2020*).

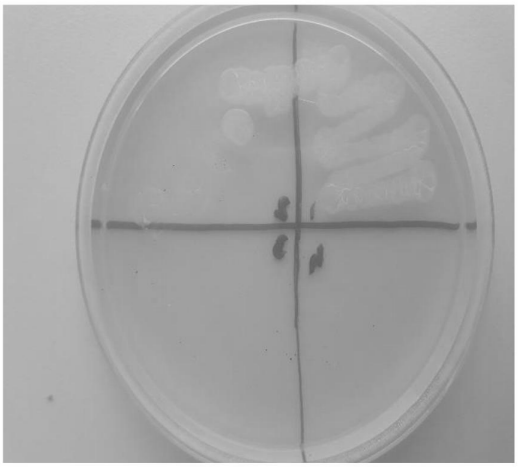


Figure 17. White, cloudy, and circular *E. coli* colonies observed on MUG agar plates. (*Photograph by author, taken on November 21, 2020*).

A streak plate was made by transferring the *E. coli* colony to MUG agar plate and streaking it across the quadrants using sterile techniques. The streak plate was then incubated at 37^0 C for 24 hours. The next day, large, white, cloudy, and circular *E. coli* colonies were observed on the first and second quadrant of the MUG agar plate (Figure 17). A single *E. coli* colony was selected from the MUG agar plate with an inoculating loop and was transferred to

a sterilized flask with 60 mL of freshly prepared LB broth. The inoculated LB broth was then incubated at 37^0 C for 24 hours, after which dilution analysis was done to determine which serial dilution factor of the inoculated broth will result in countable *E. coli* numbers that can be used in bacteriological analysis.

Serial dilution analysis was conducted following the methods described in the section 5.3.4 of this thesis. Enumeration of *E. coli* numbers through IDEXX Colilert-18 Test/Quanti-Tray/2000 Method revealed that the serial dilutions of 10^{-2} and 10^{-4} each had 2419.6 MPN of *E. coli* per 100 mL and the serial dilutions of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} showed <1 MPN *E. coli*/ 100 mL of sample. 2419.6 MPN is the highest number of *E coli* that can be detected by IDEXX Colilert-18. The serial dilution analysis was conducted the second time using potassium dihydrogen phosphate buffer solution to ensure the bacteria cells were not being lysed by milliQ water. But all the serial dilutions greater than 10^{-4} again showed <1 MPN *E. coli*/ 100 mL of sample.

However, the IDEXX trays displayed fluorescent-coloured wells after three days of incubating them. Since colilert-18 media is selective, only wells that are positive (with fluorescent wells) after 24-28 hours of incubating the IDEXX trays can be confirmed as wells with *E. coli* growth (IDEXX® Laboratories 2013). Fluorescent IDEXX wells observed after three days of conducting serial dilution analysis indicated that the bacterial growth on MUG agar plates did not consist of pure *E. coli* colonies. Inability to collect pond water the second time due to ongoing winters led to choosing cultured *E. coli* strains for the bacteriological research.

Appendix B. Documentation of the research through photos

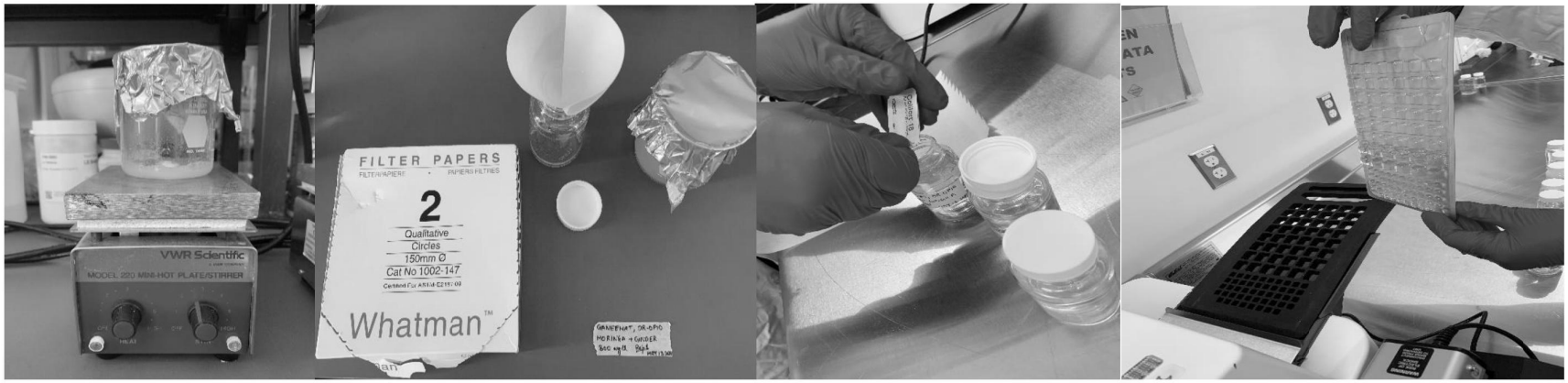
(Left to right) *Moringa oleifera* seeds that were grown in the EFL greenhouse at UNBC; Preparation of soil mixture by adding adequate quantities of peat, coarse sand, coir, slow-release nutrients, and dolomite; Adding freshly prepared soil mixture in one-litre pots. (*Photographs by author*).

(Left to right) After sowing the *Moringa* seeds on February 27, 2019, the pots received light watering; The *Moringa* seeds showed 86% germination success; The seedlings were transplanted into larger four-litre pots on May 2, 2019. (*Photographs by author*).

(Left to right) *Moringa* seedlings during the last week of April 2019; the *Moringa* plants during the first week of July 2019; *Moringa* plants during the last week of August 2019; A harvested *Moringa* root on September 27, 2019. (*Photographs by author).*

(Left to right) The harvested, washed and peeled *Moringa* and ginger roots were freeze-dried using Labconco FreeZone[6] freeze dryer in the beginning of June 2020; The freeze-dried roots were cut into smaller pieces using a knife and a wooden board; The freeze-dried roots were ground into a fine powder using Black+Decker coffee and spice grinder; Composite samples of *Moringa* and ginger roots were created by mixing the root powders of different *Moringa* and ginger plants respectively. (*Photographs by author*).

(Left to right) A thawed ATCC® mini-cryovial containing 8739™ *E. coli*; VWR's powdered LB Broth (Luria-Bertani) used for preparing LB broth; Criterion's dehydrated culture media of MUG nutrient agar used for preparing MUG agar plates; Comparison of sterile LB broth with inoculated LB broth; A streak MUG agar plate with *E. coli* colonies. (*Photographs by author).*



(Left to right) Appropriate quantities of *Moringa* and ginger root powders were added to 10mL buffered milliQ water and stirred for 15 minutes on VWR mini hot-plate stirrers using magnetic stir bars; After adding *Moringa* and ginger root powders to 100ml milliQ water with standardized *E. coli* numbers, the treatments were filtered into sample bottles through Whatman 150 mm, grade 2 filter paper; After filtering the treatments, Colilert-18 reagent was added to each sample bottle; The treatments were then vigorously shaken and poured into sterile Quanti-Trays/2000. (*Photographs by author*).

(Left to right) Quanti-Trays were sealed using IDEXX Quanti-Tray™ Sealer; Sealed Quanti-Trays were stacked and placed in the incubator at 37^0 C for 24 hours; After 24 hours, the Quanti-Trays were viewed under UV light and the number of fluoresced wells were counted and the information was compared with IDEXX MPN table to determine MPN of *E. coli* present in each Quanti-Tray. (*Photographs by author*).