

**ETHNOBOTANY AND BIOACTIVITY OF MEDICINAL PLANTS USED TO
TREAT SYMPTOMS ASSOCIATED WITH GASTRO-INTESTINAL
INFECTIONS IN NAMIBIA**

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ABSTRACT

The goal of the study was to determine the plants used in Namibia to treat symptoms linked to gastro-intestinal tract infections (GITI) and to verify their antimicrobial activity. An ethnobotanical survey was conducted to document plants used in Namibia to treat symptoms related to GITI, and eighteen plant species (from 16 families) were collected and identified. Aqueous and organic extraction methods were used to obtain crude extracts that were tested on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Shigella sonnei* by disk diffusion method. Lastly, fractionation by vacuum liquid chromatography was done on extracts of *Terminalia sericea* and *Ximenia caffra* and tested against *E. coli*, *S. aureus* and *S. sonnei*. Of these plants, *T. sericea* and *Harpagophytum procumbens* and *Aloe sp.* were the most mentioned plants used during the ethnobotanical survey. Some of the crude extracts tested had no inhibitory effect on any of the tested bacteria. Of the crude aqueous extracts tested, *X. caffra* and *Moringa oleifera* had the highest activity against *B. subtilis*, while *H. procumbens* and *Parinari capensis* were highly active against *E. coli*. Crude organic extracts from *T. sericea* and *X. caffra* showed the highest inhibitory effects against *E. coli*, *S. aureus* and *S. sonnei* and *Acacia erioloba* and *Aloe sp.* on *B. subtilis*. Furthermore, 20% methanol in Dichloromethane (DCM) and 10% acetone (in DCM) fractions from *T. sericea* had the most activity against *S. aureus* and *S. sonnei* respectively. The 100% methanol fraction and 10% acetone (in DCM) fraction from *X. caffra* were most effective against *S. aureus* and *S. sonnei*, respectively. Minimum inhibitory concentration of *T. sericea* root extract was lowest against *S. aureus* (0.1 mg/mL) and *S. sonnei* (1 mg/mL). From the results obtained, it is plausible that plants tested can be effective in treating some infections associated to GITI and are effective against both gram-positive and gram-negative microorganisms. These results show that the objectives were met. Additionally, as the most effective fractions were from *T. sericea* and *X. caffra*, conducting further research to determine the active compounds may be necessary.

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LIST OF ABBREVIATIONS

ATR	-	Acid Tolerance Response
EAEC/EaggEC-		Enterοaggregative <i>E. coli</i>
EIEC	-	Enterοinvasive <i>E. coli</i>
EPEC	-	Enterοpathogenic <i>E. coli</i>
ETEC	-	Enterοtoxigenic <i>E. coli</i>
DCM	-	Dichlorοmethane
DMSO	-	Dimethyl Sulfoxide
GIT	-	Gastro-intestinal Tract
GITI	-	Gastro-intestinal Tract Infection
IK	-	Indigenous Knowledge
MALT	-	Mucosa-associated Lymphoid Tissue
MIC	-	Minimum Inhibitory Concentration
MOHSS	-	Ministry of Health and Social Sciences
NBRI	-	National Botanical Research Institute
NMEC	-	Neonatal Meningitis <i>E. coli</i>
NRPS	-	Non-Ribosomal Peptide Synthetase
NSP	-	Non-starch Polysaccharides
NTS	-	Non-typhoidal <i>Salmonella</i>
ORS	-	Oral Rehydration Salts
ORT	-	Oral Rehydration Therapy
PUD	-	Peptic Ulcer Disease
SCV	-	<i>Salmonella</i> Containing Vacuole
SE	-	Standard Error
STEC	-	Shiga Toxin-producing <i>E. coli</i>
UNICEF	-	United Nations Children’s Fund
VLC	-	Vacuum Liquid Chromatography
WGO	-	World Gastroenterology Organization
WHO	-	World Health Organization

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DECLARATION

I, Lisanza Therese Mulyangote, hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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Date.....

Lisanza T. Mulyangote

CHAPTER 1: INTRODUCTION

1.1. Background

Gastroenteritis and peptic ulcers are gastro-intestinal tract infections (GITI) commonly caused by microorganisms, spread through the faecal-oral route by ingesting food or contaminated with human or animal faeces (Madigan *et al.*, 2012; Bauman, 2015). Lack of access to water facilities worldwide makes it more difficult to control this infection which causes diarrhoea as a common symptom (Oppong *et al.*, 2015). Many people use medicinal plants to treat numerous ailments.

1.1.1. Gastroenteritis

Gastroenteritis is defined as an inflammation of the stomach or the intestines, caused by the presence of bacteria, viruses or parasites (Fhogartaigh and Dance, 2013; Bauman, 2015). The symptoms associated with gastroenteritis include abdominal pain, diarrhoea, nausea and vomiting (Bauman, 2015). The major clinical sign, diarrhoea, has two basic mechanisms, which are through the production of toxins called enterotoxins and through the damage of the intestinal epithelium (Gilligan, Shapiro and Miller, 2014).

With viral gastroenteritis, the most common cause of infection are caliciviruses, astroviruses and rotaviruses, whereas gastroenteritis caused by protozoans are commonly caused by *Giardia intestinalis*, *Cryptosporidium parvum* and *Entamoeba histolytica* (Bauman, 2015). Several pathogens are responsible for bacterial gastroenteritis, and six of the most common microorganisms are *Shigella* sp., *Campylobacter* sp., *Clostridia* sp., *Salmonella* sp., *Escherichia coli* and *Vibrio cholerae*. (Tille, 2014).

1.1.2. Food poisoning

Food poisoning is also known as food intoxication and is a disease that results from ingesting food that contains performed microbial toxins (Madigan *et al.*, 2012). Staphylococcal food poisoning is caused by toxins produced by *Staphylococcus aureus*. This bacteria is able to cause infection as it can grow in many common foods, and it is able to produce heat-stable enterotoxins. Infection starts between 1-6 hours of food consumption that has been infected with *S. aureus*, leading to symptoms such as vomiting, nausea, and diarrhoea (Tille, 2014). Other microbes that cause food poisoning are *Bacillus cereus* and *Clostridium perfringens*. They also produce exotoxins that cause vomiting and diarrhoea and are sporulating gram-positive bacteria (Cowan, 2012; Madigan *et al.*, 2012).

B. cereus food poisoning is usually associated with consumption of cooked meat or vegetables that are kept at warm temperatures for long periods of time, conditions that favour production of heat-stable exotoxins (Cowan, 2012). When one consumes contaminated animal flesh like meat or fish and vegetables such as beans that have not been cooked thoroughly enough to destroy endospores, they are at a high risk of infection by *C. perfringens* (Cowan, 2012; Madigan *et al.*, 2012).

1.1.3. Peptic Ulcer Disease (PUD)

Peptic ulcers is defined as an erosion of the lining of either the stomach, leading to gastric ulcers or the duodenum of the small intestine, which causes duodenal ulcers (Allué, 2015; Bauman, 2015). The causative agent, *Helicobacter pylori* is known to infect about 50% of the world's population, with a prevalence of about 30-40% in developed countries, and over 80% in developing countries (Allué, 2015). About 90% of peptic ulcer disease (PUD) cases are caused by *H. pylori* (Fleming 2007). about 5 - 10% of them develop ulcerations (Sullivan, 2010). *H. pylori* infections may also cause gastritis, stomach cancer, adenocarcinoma and mucosa-associated lymphoid tissue lymphomas, such as MALT lymphoma (Allué, 2015). PUD associated with *Helicobacter pylori* infections usually cause 95% of gastric or stomach ulcerations and 70% of duodenal ulcerations.

Symptoms related to PUD are subtle and most people are known to be asymptomatic, making misdiagnosis and treatment failure common. Although that may be possible, in cases where an ulcer has developed, symptoms are different for uncomplicated and complicated PUD (Oppong *et al.*, 2015). Uncomplicated symptoms include epigastric pain, nausea, vomiting and weight loss, whereas complications include the ulcers healing and relapsing spontaneously, the ulcer may also bleed leading to haematemesis and anaemia, and perforations may lead to peritonism (Oppong *et al.*, 2015). Due to the fact that management of the disease has become complicated, the use of medicinal plants as a form of treatment have been considered (Orsi *et al.* 2012).

1.2. Orientation of the study

Since information about medicinal plants used to treat GITI in Namibia has not been documented, the direction that the study took, was first of all be to conduct an ethnobotanical study. This facilitated in providing data that may be available, but is unknown to the public. The second path the study took was to test the antimicrobial activity of the products against the bacterium. This could help in understanding the use of the medicinal plants and how they are used in treating GITI.

1.3. Statement of the Problem

Treating symptoms related to GITI has been challenging as drugs are only administered when an individual is infected with *Salmonella* sp. and in other cases, only oral rehydration therapy is administered. In cases on *H. pylori* infection, a triple drug therapy is administered, but emerging resistance has complicated the treatment. Medicinal plants are used by various communities in Namibia to treat symptoms related to GITI. However, there is anecdotal information on the medicinal plants used to treat symptoms related to GITI in Namibia. Furthermore, the antimicrobial properties of these plants for GITI symptoms treatment have not been studied in Namibia.

1.4. Objectives

The objectives of the study are:

- a) To document ethnobotanical medicinal plants used in the treatment of gastro-intestinal infections in Namibia.
- b) To evaluate antimicrobial properties of these medicinal plants.

1.5. Significance of the Study

The significance of the study is to be able to have documentation of medicinal plants used to treat GITI in Namibia. The data obtained from the activity of the plant extracts can lead to drug development. Furthermore, as the data on the active fractions of the plants are anecdotal, the study will attempt to isolate these active fractions and determine their activity. Lastly, this study will aid at improving the health care of people by possibly providing a cheaper alternative method of treatment.

1.6. Limitations of the Study

One limitation of the study is that indigenous knowledge (IK) data obtained during the interview from individual sources may have weaknesses in terms of validity and reliability. Limitations in accuracy, and variability of data may also be a restriction in the study. The number of samples collected and the study sites were small and this could have caused confines within the study. Another drawback is the lack of data on dosages. This is because the respondents preferred to keep this information to themselves. As cytotoxicity was not done in the study, cellular safety of the plants is not known.

CHAPTER 2: LITERATURE REVIEW

2.1. Epidemiology of Gastro-intestinal Tract Infections

Gastro-intestinal tract infections are known to occur in both developed and developing countries, but occur more in developing countries. This is because they are usually associated with poor sanitation, as they are mostly spread by faecal-oral transmission. Infectious diarrhoea as a symptom of GITI is a global public health problem with high mortality and morbidity, especially in children in developing countries (Verdu and Riddle, 2012).

Globally there has been a large decline in mortality rates amongst children dying from diarrhoea, with a decline from 146 per 1000 in 1970 to 79 per 1000 in 2003, but unfortunately in Africa, it shows the smallest reductions in mortality rates and is the most marked slowing down trend (Karambu *et al.*, 2013). Karambu *et al.* (2013) states that the under-five mortality rate in the African continent is seven times higher than in the European region. It has been approximated that 88% of all diarrheal diseases are as a result of contaminated water and inadequate hygiene and sanitation (Karambu *et al.*, 2013).

2.2. Global burden

According to the World Health Organization (WHO) and The United Nations Children's Fund (UNICEF), there are in the region of two billion cases of diarrhoeal diseases worldwide every year, with 1.9 million deaths occurring in children below the age of 5 years, mostly in developing countries (World Gastroenterology Organization

[WGO] 2012). This means that about 18% of all deaths of children younger than 5 years of age are due to diarrhoeal illnesses, and 78% of these deaths occur in Africa and South-East Asia (WGO 2012). It has also been estimated based on empirical modelling of active, passive and outbreak surveillance data in the United States of America, that 31 major pathogens caused 9.4 million episodes of diarrhoeal illnesses, 55, 961 hospitalizations and 1, 351 deaths (Verdu and Riddle, 2012).

Salmonella, which causes salmonellosis diarrhoea, is said to be a huge contributor to morbidity and mortality worldwide, causing about 93 million cases and 155 000 deaths each year (Williams *et al.*, 2016). It has also been estimated that 91 million of individuals worldwide contract shigellosis each year (Gu *et al.*, 2012). Diarrhoea as a symptom of GITI is the second leading killer of children, and approximately one in five children under the age of five die as a result of dehydration, weakened immunity or malnutrition associated with diarrhoea (Karambu *et al.*, 2013). In the year 2000, 21.9% of all deaths of children under the age of 5 in Sub-Saharan Africa were due to diarrhoea (Karambu *et al.*, 2013).

2.3. Burden in Africa

Cases of diarrhoea remain high in sub-Saharan Africa for children under the age of five. Although the mortality rate has been decreasing yearly by about 4% since 2000, mortality remains high, and diarrhoeal diseases contribute 12% of the estimated 3.6 million of child deaths in 2010 (Langendorf *et al.*, 2015). Throughout the African continent, incidence of childhood diarrhoea has reduced from 4.2 to 3.3 episodes per child-year from 1990 to 2010, with Sub-Saharan Africa accounting for a third of

diarrhoeal episodes yearly (500 million of 1.7 billion worldwide), and most of these incidents occur in children between the ages six to eleven months (Langendorf *et al.*, 2015).

In resource-poor settings of Africa, enteric fever is a public health concern with an incidence of 10-100 per 100,000 cases per year (Tadesse, 2014). About 42% of deaths in children worldwide caused by dehydration, weakened immunity or malnutrition associated with diarrhoea occur in the WHO African region (Karambu *et al.*, 2013). A study in Morocco in 2011 showed that 132 000 children less than 5 years of age were reported to have different degrees of dehydration associated with diarrhoea, of which approximately 17.4% were from the Rabat-Sale-Zemmour-Zair region, and 75.5% from urban areas (Benmessaoud *et al.*, 2015).

2.4. Burden in Namibia

In Namibia, the epidemiology of GITI is not well documented. In the year 2013 an outbreak of cholera occurred in Opuwo District of the Kunene Region. This led to the death of 11 people and total of 316 cases (The United Nations Children's Fund, 2014; World Health Organization, 2014).

Despite being the only documented case, there are studies in Namibia that give an account of GITI causative agents being found in animals and animal products. One such study has reported that 0.85% beef samples in Namibia are infected with *Salmonella* (Shilangale *et al.*, 2012). A total of twenty nine *Salmonella* serovars were identified with *S. Chester* being the most frequent isolated, followed by *S.*

Schwarzengrund and *S. Chartres* (Shilangale *et al.*, 2012). A wide distribution of salmonellosis has also been observed in Namibia in several animals, including cattle between the years 1990 and 2009 (Magwedere *et al.*, 2012). Furthermore, shiga toxin-producing *E. coli* serovars have been isolated from sheep in Namibia (Madzingira, 2016). Zoonosis, known as the transfer of diseases from animals to humans, account for about 75% of emerging human infections in humans worldwide (Magwedere *et al.*, 2012). It has been seen that animals possess some of the most common causative agents of GITI in Namibia, making easy transfer from these animals to humans is possible. This will likely lead to an increase in cases in Namibia.

2.5. Gastro-intestinal Tract

The gastro-intestinal tract (GIT) is a long tube and consists of the mouth, oesophagus, stomach, intestines, rectum and anus, and is responsible for the digestion of food, absorption of nutrients, and the production of nutrients by the indigenous microbial flora (Madigan *et al.*, 2012; Bauman, 2015). The acidic stomach fluid, which is at a pH of about 2 is said to serve as a chemical barrier, preventing the entry of microorganisms into the GIT (Madigan *et al.*, 2012).

Even though this is the case, there are still microorganisms that are found in the different parts of the tract, and include species of gram-positive bacteria, *Fusobacteria*, *Actinobacteria*, *Proteobacteria* and *Bacterioidetes* (Madigan *et al.*, 2012; Tille, 2014) (**Table 1**). Aerobes, which includes Enterobacteriaceae such as *E. coli*, *enterococci* and *streptococci* are normally outnumbered by anaerobes (Tille, 2014), and the most common single organism that is found is *H. pylori*, which is known to colonize the

stomach wall in many individuals and may cause ulcers in susceptible individuals (Madigan *et al.*, 2012).

Within the intestinal tract, which consists of the small intestine and large intestine, the microflora in humans varies and is to some extent based on the individual's diet (Madigan *et al.*, 2012). The small intestine is divided into two parts, the duodenum and the ileum, which are joined by the jejunum, of which the ileum and jejunum have a mucosa that allows penetration of toxins (Bannister, Gillespie and Jones, 2006). The duodenum has similar microflora to the stomach because it is fairly acidic. Moving further down from the duodenum to the ileum, the pH increases and the number of bacteria increase (Madigan *et al.*, 2012).

The large intestine is known to possess a large amount of bacteria, including facultative aerobes such as *E. coli*, as well as gram-positive and gram-negative rods, cocci and sporing organisms (Bannister, Gillespie and Jones, 2006; Madigan *et al.*, 2012). These aerobes tend to consume the remaining oxygen found in the colon of the large intestine, which provides a suitable condition for the growth of obligate anaerobes such as *Clostridium* and *Bacteriodes* (Bauman, 2015). It is important to know that not all microbes of the GIT are harmless. There are numerous bacteria, fungi, viruses, protozoa, and parasitic worms that cause diseases of the digestive system (Bauman, 2015).

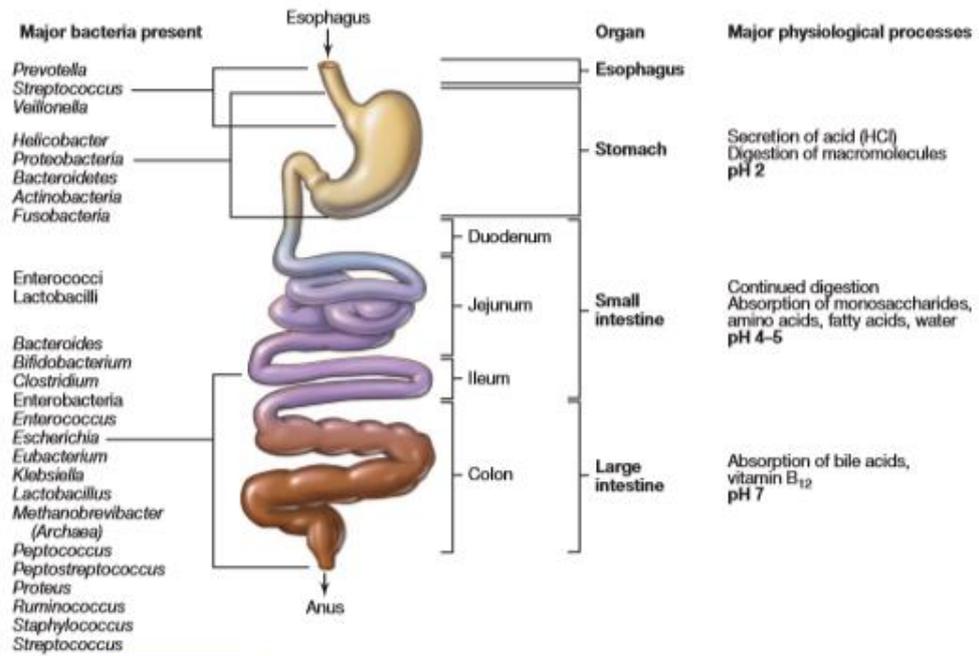


Figure 1: The human gastro-intestinal tract. The distribution of representative non-pathogenic organisms often found in healthy adults Source: Madigan *et al.* (2012).

2.6. Causative agents

As mentioned above, there are many microorganisms that cause GITI, including bacterial species. These include *E. coli*, *S. aureus*, *B. cereus*, *Shigella sp.*, *Salmonella sp.*, *H. pylori*, and *V. cholera*, of which some of them are explained in detail below. In general all these bacteria cause the same symptoms in the infected individual and Fhogartaigh and Dance (2013) state that there are three main mechanisms by which bacteria cause gastroenteritis, which is shown in **Table 1**.

Table 1: Mechanisms by which bacteria cause gastroenteritis and the associated conditions

Mechanism	Syndromes
Production of preformed toxins	Induces vomiting and abdominal cramps within a few hours of ingestion
Secretion of toxins after adhering to the intestinal epithelium	Watery diarrhoea, without blood or mucus or associated fever (non-inflammatory)
Invasion of the intestinal mucosa	Causes dysentery, the passage of small-volume stools containing blood, mucous, and pus associated with fever, low abdominal pain and tenesmus (inflammatory)

2.6.1. *Escherichia coli*

Escherichia coli is one of the common microbes that cause bacteria gastroenteritis, and it is also known to be the most common cause of traveller’s diarrhoea (Bauman, 2015). This microorganism is part of a group of bacteria known as coliforms, that can be aerobic or facultatively anaerobic (Madigan *et al.*, 2012). Not only can they be found in the colon of humans and animals, coliforms can also survive in soil, plants, decaying vegetation, and in contaminated water (Bauman, 2015). The antigens, O157, O111, H8 and H7 are used to describe the different strains of *E. coli*, and are associated with virulence (Bauman, 2015).

At least five groups of pathogenic *E. coli* strains cause a range of gastro-intestinal ailments (Fhogartaigh and Dance, 2013). These groups include Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enterohaemorrhagic/shiga toxin-producing *E. coli* (STEC), neonatal meningitis *E. coli* (NMEC) and Enteroaggregative *E. coli* (EaggEC) (Bisi-Johnson *et al.*, 2011;

Fhogartaigh and Dance, 2013). Each group of diarrhoeagenic *E. coli* has virulence factors that enable it to cause infection. **Table 2** shows what these virulence factors are and how they cause infection.

Table 2: Pathogenesis and spectrum of disease of diarrhoeagenic *E. coli*

Organism	Virulence factors	Spectrum of Disease and infections
ETEC	Pili that permits gastro-intestinal colonization. Heat-labile (LT) and heat-stable (ST) enterotoxins that mediate secretions of water and electrolytes into the bowel lumen	Traveller's and childhood diarrhoea, characterized by profuse, watery stools. Transmitted by contaminated food and water
EIEC	Virulence factors uncertain, but organisms invade enterocytes lining the large intestine in a manner nearly identical to <i>Shigella</i>	Diarrhoea in infants in developing, low-income nations; can cause a chronic diarrhoea
EPEC	Bundle-forming pilus, intimin, and other factors that mediate organism attachment to mucosal cells of the small bowel, resulting in changes in cell surface	Diarrhoea in infants in developing, low-income nations; can cause a chronic diarrhoea. Transmitted from person to person as a result of overcrowded, unhygienic conditions and through ingestion of contaminated food.
STEC	Toxin similar to Shiga toxin produced by <i>Shigella dysenteriae</i> . Most frequently associated with certain serotypes, such as <i>E. coli</i> O157:H7	Inflammation and bleeding of the mucosa of the large intestine. Can lead to haemolytic-uremic syndrome, resulting from toxin-mediated damage to kidneys. Transmitted by ingestion of undercooked meat or unpasteurized milk
EaggEC	Involves binding by pili, ST-like and hemolysin-like toxins. Actual pathogenic mechanism is unknown	Chronic diarrhoea in children in developing countries and traveller's diarrhoea

Source: Tille (2014).

2.6.2. *Shigella* sp.

Another form of bacterial gastroenteritis is shigellosis, which is caused by the genus *Shigella*. The symptoms associated with this form of gastroenteritis is similar to the others and consists of a fever, abdominal cramps, diarrhoea, and sometimes bloody stool and vomiting (Cowan, 2012; Bauman, 2015). As the symptoms are the same for all forms of gastroenteritis, one can only determine which organism is causing infection by conducting laboratory tests from stool samples collected from the patient. The four species that cause infection include *Shigella flexneri*, *S. sonnei*, *S. dysenteriae* and *S. boydii*, of which *S. sonnei* and *S. flexneri* are the most causes of infection (Bauman, 2015).

Just like *Salmonella*, *Shigella* sp. have type III secretion systems that they use to release diarrhoea-producing enterotoxins into the host cell. These enterotoxins bind to the surface proteins on the epithelial cells lining of the intestines, which leads to loss of electrolytes and water. *S. dysenteriae* also secretes Shiga toxin, which is an exotoxin that stops protein synthesis in the host's cells, and results in a more severe form of shigellosis with a mortality rate as high as 20% (Bauman, 2015).

Infection by this bacterium is caused by ingestion of contaminated food, mostly chicken eggs, and water (Cowan, 2012). Once ingested, the bacteria passes through the stomach and attaches to the lining of the small intestine, inducing endocytosis, and eventually killing host cells (Bauman, 2015). This initiates a fever, abdominal cramps and diarrhoea, which are the symptoms associated with gastroenteritis (Bauman, 2015). Other symptoms that occur with *Salmonella* infection include vomiting and in

severe conditions, blood in the stool (Cowan, 2012). The virulent serotypes of *Salmonella* have the ability to tolerate low pH levels of the stomach, which allows them to survive in the stomach, and on top of that, they have adhesins, making it possible for them to attach to the cells, and type III secretion systems that introduce toxins (Cowan, 2012; Bauman, 2015). A type of bacterial adhesins are bacterial lectins which occur commonly in the form of elongated, submicroscopic, multisubunit protein appendages, which interact with glycoproteins and glycolipid receptors of host cells using fimbriae or pili (Esko and Sharon, 2009).

These toxins disrupt mitochondria, inhibit phagocytosis, rearrange the cytoskeletons of the eukaryotic cells or induce apoptosis (Bauman, 2015). *Shigella* invades the villus cells of the large intestine, and unlike *Salmonella*, does not perforate the intestine or invade the blood (Cowan, 2012). It infects the intestinal mucosa of the large intestine initiating an inflammatory response that causes extensive tissue destruction. The bacterium also releases endotoxins that cause fever. Enterotoxin causes damage to the mucosa and villi, which gives rise to bleeding and secretion of mucous where the erosion occurred. A heat-labile exotoxin known as shiga toxin is also released, and causes more damage to the intestine in addition to systemic effects such as injuring the nerve cells.

2.6.3. *Salmonella* sp.

Salmonella sp. are gram-negative bacteria that are known to cause two disease conditions, typhoid fever and a form of gastroenteritis referred to as salmonellosis (Bauman, 2015). This motile genus is known to inhabit the intestines of almost all

vertebrates and are eliminated in their faeces (Cowan, 2012). More than 2000 unique strains of *Salmonella* have been identified by analysing the DNA sequence, and this has shown that they all belong to the species *S. enterica* (Bauman, 2015).

Although this is the case, the historical names are used by medical people and researchers. The serotypes Enteritidis and Typhimurium are known to be responsible for most of the causes of human salmonellosis in the United States of America (Bauman, 2015). Non-typhoidal *Salmonella* (NTS) has been found to be the dominant contributor to invasive bacterial disease with high mortality in Africa (Feasey *et al.*, 2012; Langendorf *et al.*, 2015).

Similar to other microbes that cause GITI, *Salmonella* infection begins when one ingests food or water contaminated with the organism. These organisms have several virulence factors that allow them to overcome the obstacles within the gut. First of all, *Salmonella* sp. activate the acid tolerance response (ATR), which allows them to maintain their pH at a value higher than their environment, thereby preventing severe acid shock (Fàbrega and Vila, 2013).

Secondly, in the small intestine, *Salmonella* is able to evade the intestinal mucous layer by entering the M cells of the Peyer's patches (PPs) in the intestinal epithelium (Fàbrega and Vila, 2013). After the bacteria adheres to the epithelium, responses from the host cause the bacteria to be engulfed by large vesicles called *Salmonella*-containing vacuoles (SCVs), where the bacteria is able to survive and replicate (Fàbrega and Vila, 2013). During this process, they illicit an immune response which are responsible for inflammation.

Apart from ATR and the ability to alter phagocytosis so as to circumvent the process, *Salmonella* has other factors that enable it to invade the intestinal epithelium, such as fimbriae, which plays a role in adhesion and invasion (Bisi-Johnson *et al.*, 2011), flagella, which allows it to approach the intestinal epithelium and access nutrients in the intestine (Fàbrega and Vila, 2013). The flagella has an H antigen, which also enhances adherence, and other structural bodies, fimbriae and the cell surface polysaccharide O antigen does the same (Madigan *et al.*, 2012). Iron-chelating siderophores contribute to the virulence of *Salmonella* by sequestering iron to aid in growth (Madigan *et al.*, 2012). Three toxins, cytotoxins, endotoxins and enterotoxins produced by this bacterium also contribute to the virulence by killing the host cells by inhibiting protein synthesis and allowing calcium ions to escape (Madigan *et al.*, 2012) (Figure 2).

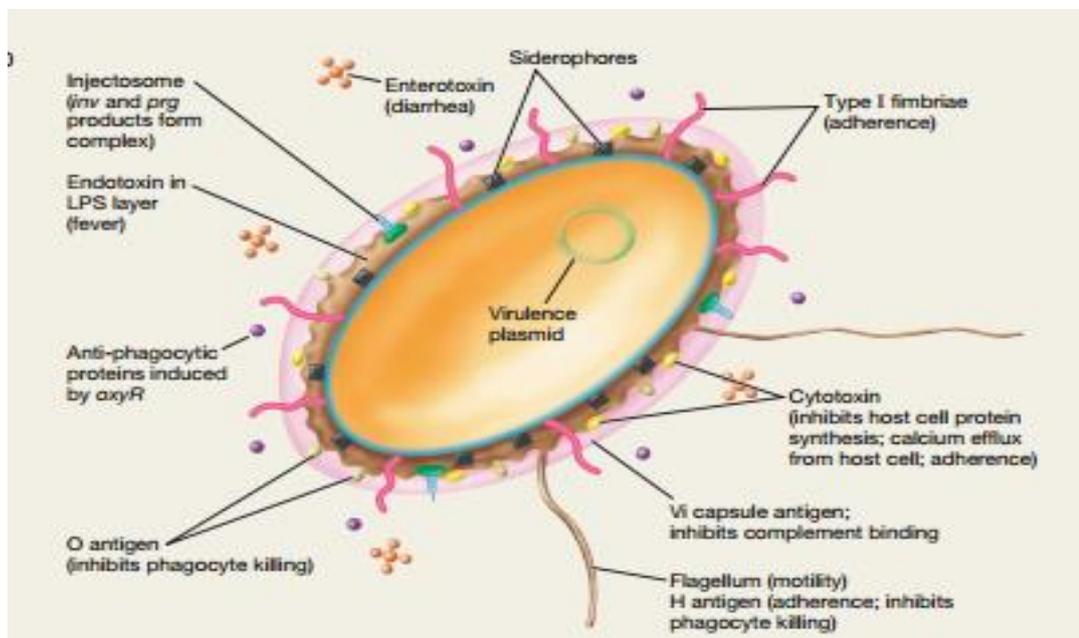


Figure 2: Virulence factors in *Salmonella* pathogenesis (Source: Madigan *et al.* (2012)).

2.6.4. *Staphylococcus aureus*

Staphylococci are nonsporulating gram-positive cocci about 8 – 10 µm in diameter (Tille, 2014). They may be present as part of the normal microflora of humans and animals, with 30% of healthy humans carrying *S. aureus* on the skin and nasal cavities (Rajkovic, 2016). Although *S. aureus* normally causes pus-forming infections such as boils and impetigo, this bacterium also produces toxins that cause foodborne illnesses (Madigan *et al.*, 2012). Foodborne illnesses by *S. aureus* exotoxins are associated with eating certain foods, such as custards, sauces, cream pastries, processing meats, chicken salads or ham that have been contaminated by handling and then left unrefrigerated for a few hours (Cowan, 2012).

S. aureus is an organism that not only causes food poisoning but it also causes skin infections as well as respiratory infections (Madigan *et al.*, 2012). This is because of several toxins that it possesses. Each toxin produced by *S. aureus* causes a certain action in the body, with enterotoxins being responsible for symptoms associated with Staphylococcal food poisoning (**Table 3**). Within an hour of ingestion of staphylococcal enterotoxin (SE) contaminated food, food poisoning occurs, which is characterized by severe vomiting and diarrhoea (Pinchuk, Beswick and Reyes, 2010)

Staphylococcal enterotoxin A (SEA) is the most common cause of food poisoning by *Staphylococcus* sp., and it has been suggested that its site of action is the intestine because studies have shown that there is an increase in the number of lymphocytes and polymorphnuclear cells in the jejunum and duodenum (Pinchuk, Beswick and Reyes,

2010). Pinchuk et al. (2010) states that ingestion also leads to quick emetic and neurobehavioral responses.

Table 3: *S. aureus* toxins causing disease in humans

Toxin	Action
α -Toxin (CT)	Hemolysis
Toxic shock syndrome toxin (SA)	Systemic shock
Exfoliating toxin A and B (SA)	Peeling of skin, shock
Leukocidin (CT)	Destroys leukocytes
B-Toxin (CT)	Hemolysis
γ -Toxin (CT)	Kills cells
δ -Toxin (CT)	Hemolysis, leukolysis
Enterotoxin A, B, C, D and E (SA)	Induce vomiting, diarrhoea, shock
Coagulates (E)	Induces fibrin clotting

AB toxin; CT, cytolytic toxin; E, enzymatic virulence factor; SA, superantigen toxin

2.6.5. *Bacillus* sp.

Bacillus was first identified in the year 1920 as a genus of gram-positive, aerobic spore formers (Logan, 2012). *Bacillus cereus* is a sporulating gram-positive bacterium that is naturally present in soil, and so is commonly found on vegetables and other products in close contact with soil (Cowan, 2012). The toxins produced by *B. cereus* are able to cause diarrhoeal and emetic symptoms (Logan, 2012). The emetic syndrome manifests as nausea and vomiting within one to five hours after the consumption of contaminated food, while diarrhoeal syndrome includes abdominal pain, profuse watery diarrhoea, and occasionally nausea and vomiting and typically occurs eight to sixteen hours after contamination (Castiaux *et al.*, 2015).

Another species, *B. subtilis* has also been implicated in foodborne illnesses, causing vomiting as the most common symptom, which would sometimes be accompanied by diarrhoea (Logan, 2012). *B. subtilis* together with *B. licheniformis* and *B. pumilus* have also been associated with foodborne poisoning but to a much lesser extent than for *B. cereus* as outbreaks are not as much as those associated with *B. cereus* infection (Carlin, 2016).

As *B. cereus* is the most studied species known to cause food poisoning characterised by diarrhoea, its virulence factors will be explained. Within eight to sixteen hours of ingesting food contaminated with either spores or vegetative cells, an infected individual begins to experience symptoms related to *B. cereus* infection (Logan, 2012). Three enterotoxins, haemolysin BL (Hbl), nonhaemolytic enterotoxin (Nhe) and cytotoxin K (CytK) produced by this bacterium are associated in diarrhoeal illness and cereulide is said to cause emetic illness (Logan, 2012; Jeßberger *et al.*, 2014; Carlin, 2016). Other toxins said to be associated with diarrhoeic syndrome are enterotoxin FM (EntFM), enterotoxin S (entS), enterotoxin-T (bceT) and pore-forming haemolysins like the cereolysin O (CerO) (Castiaux *et al.*, 2015).

Cereulide is a dodecadepsipeptide that is synthesised enzymatically using Non-Ribosomal Peptide Synthetase (NRPS), and is said to function by binding to 5-hydroxytryptamine 3 (5-HT₃) receptors, thereby causing vomiting (Castiaux *et al.*, 2015). Studies conducted on other species, *B. mojavensis*, and *B. subtilis* have implicated these organisms in emetic illnesses, with a nonprotein toxin known as amolysis. In addition studies have shown that large consumption of these organisms

cause infection (Logan, 2012). *B. mojavensis* also produces cytotoxic, surfactin-like, heat-stable cyclic lipopeptide putative emetic character.

2.6.6. *Listeria* sp.

Listeria is a gram-positive, rod shaped bacterium that can be found in different environments, such as soil, silage, water, food and food processing environments (Azizoglu and Kathariou, 2016). They are also motile and facultative anaerobes that are up to 2µm in length (Schoder, 2016). This genus of non-spore formulating bacteria contains fifteen species that are divided into four clades (Azizoglu and Kathariou, 2016). Species in clade I includes *L. monocytogenes*, *L. welshimeri*, *L. innocua*, *L. seeligeri*, which are usually isolated from food and food-related environments, as well as *L. ivanovii* and *L. marthii*, which are found in other environments (Azizoglu and Kathariou, 2016). Both processed and unprocessed foods such as raw meat, fish, cheese and ice cream can be contaminated with *L. monocytogenes* (D'Ostuni *et al.*, 2016).

Of all the species, *L. monocytogenes* is the only bacterium that is pathogenic to humans and other vertebrates such as birds (Mogomotsi and Chinsebu, 2012; Azizoglu and Kathariou, 2016). Infection by *L. monocytogenes* causes non-invasive gastro-intestinal illnesses in immunocompetent people, and invasive listeriosis infections that can lead to septicaemia and meningitis in pregnant women or immunocompromised individuals (Allen *et al.*, 2016). Symptoms associated with GITI caused by *L. monocytogenes* are non-bloody diarrhoea, nausea and vomiting (Mogomotsi and Chinsebu, 2012).

L. monocytogenes infects its host by consumption of contaminated food and this microorganism finds a way to survive and grow in its host using different virulence factors. First of all, *L. monocytogenes* is an example of a 'cystol-adapted pathogen' as it is able to invade host cells, phagocytes and nonphagocytes and then escape into the cystol, and the escape occurs before the organism matures, thereby allowing the virulent strains to survive (Schoder, 2016). The pathogenicity of *L. monocytogenes* is made possible by virulence cluster gene that is approximately 9 kb, which mediates the saprophyte-to-cytosolic parasite transition of the organism through careful regulation of the activity of the transcriptional activator protein *prfA* (Schoder, 2016). Listeriolysin O (LLO), phospholipases and *ActA* are virulence factors found in one pathogenicity island, Listeria pathogenicity island 1 (LIPI-1), on the microbes chromosome and are regulated by *prfA* (Azizoglu and Kathariou, 2016). These virulence factors play a role in surviving within the host cell and infecting neighbour cells. However, before they can be activated within the cell, two virulent factors from the internalin family, *InlA* and *InlB* are responsible for internalizing the bacteria into the host cell (Pizarro-Cerda *et al.*, 2016). *InlA* is a cell wall protein that binds to the cellular adherens junction molecule, E-cadherin, prompting internalization of *L. monocytogenes* into polarized epithelial cells and tissues, especially during transversal of the intestinal and foetal/placental barrier. *InlB* attaches to lipoteichoic acids which interact with the host molecule Met (Pizarro-Cerda *et al.*, 2016).

Once *L. monocytogenes* invades the cell, it goes into the phagosome, where the pore-forming toxin, LLO, disrupts the phagosomal compartment, releasing the bacteria into the cytosol (Azizoglu and Kathariou, 2016). It also has the ability to prevent maturation of the phagosome by preventing an increase of the pH in the phagosome.

Two membrane-active phospholipase C enzymes, PlcA and PlcB enhance rupture of the phagosomal membrane, allowing *L. monocytogenes* to escape from the phagosome to the cytosol (Azizoglu and Kathariou, 2016; Pizarro-Cerda *et al.*, 2016). Lastly, the actin polymerization protein, *ActA* facilitates intracellular motility of the microorganism and spread of the bacteria from one cell to the next (Pizarro-Cerda *et al.*, 2016).

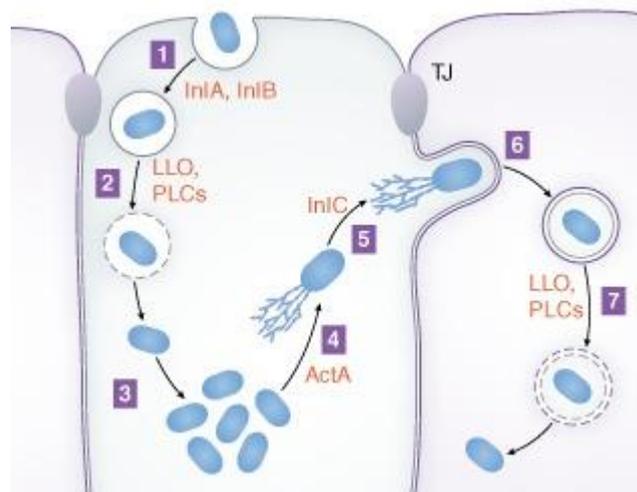


Figure 3: Intracellular life cycle of *Listeria*. Steps in the infection cycle are (1) internalization of bacteria into host cells, (2) escape from phagosomes, (3) replication in the cytosol, (4) actin-based motility, (5) formation of protrusions, (6) engulfment of protrusions, and (7) dissolution of the double membranous vacuole. The process of cell-to-cell spread comprises steps 4–7. Bacterial factors that promote various steps in the life cycle are in red lettering. ‘LLO’ denotes Listeriolysin O, and ‘PLCs’ indicates the phospholipases PlcA and PlcB. ‘TJ’ represents tight junctions Source: Ireton *et al.* (2014).

2.6.7. *Helicobacter pylori*

H. pylori is the most abundant microorganism that is located in the gastro-intestinal gut, making up over 50% of the total amount of microbes. This spiral-shaped organism was first isolated from the human stomach in 1983 and was previously known as

Campylobacter pylori, and later renamed as *H. pylori* in 1989 (Tille, 2014). *H. pylori* infections are common in majority of the world's population. Prevalence of *H. pylori* infections are higher in developing countries than in developed countries. Conditions commonly found in developing countries, such as poor sanitation, over crowded housing and low family income are factors that increase the spread of infection (Vale and Vitor, 2010). In developing countries, prevalence of *H. pylori* infection is over 80%, whereas it is as low as 40% in developed countries, (Oppong *et al.*, 2015). It is for this reason that other treatment methods should be found.

This bacterium may lead to chronic gastritis (Suzuki, Shiota and Yamaoka, 2012), and is also associated with severe gastritis-associated diseases, which include peptic ulcers and gastric cancer (Silva *et al.*, 2015). Although the exact route of transmission is unclear, possible transmission routes have been suggested. These include oral-oral, faecal-oral and a common environmental source, as well as a familial transmission (Vale and Vitor, 2010; Tille, 2014). Research studies suggest mother-to-child transmission as the most possible cause of intrafamilial spread (Tille, 2014).

Once *H. pylori* is in the GIT, it bores through the outermost mucous layer that lines the stomach epithelial tissue (Cowan, 2012). Here, it attaches to specific binding sites on the cells, allowing it to survive. This bacterium is also able to produce urease, which neutralises the acidic conditions in the stomach as it is able to convert urea to ammonia and bicarbonate. *H. pylori* uses several virulence factors to colonise the GIT. These include cytotoxin associated protein (CagA), the pathogenicity island (cag- PAI) and its effector, vacuolating cytotoxin (VacA), urease, lipopolysaccharide, peptidoglycan,

and adhesins that destroy the gastric epithelium (Morales-Guerrero *et al.*, 2012) (Figure 4).

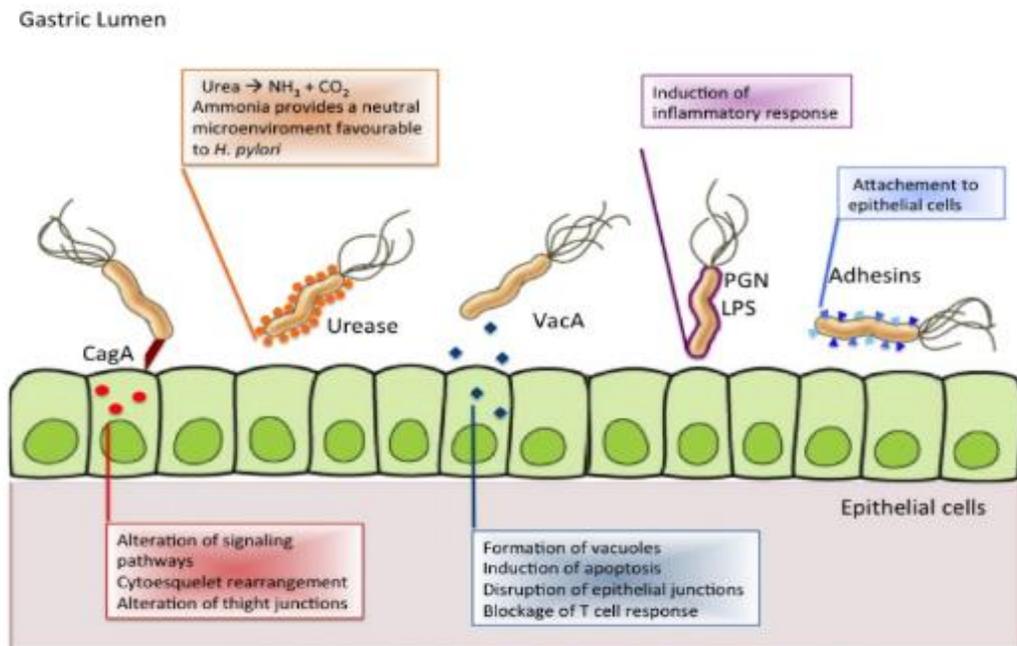


Figure 4: *Helicobacter pylori* virulence factors activities. Source: Morales-Guerrero *et al.* (2012).

2.7. Management of Gastro-intestinal Infections

2.7.1. Conventional treatment of Gastroenteritis

The main symptoms associated with GITI are diarrhoea and vomiting. Currently, there are several measures used to manage these symptoms. In cases where diarrhoea is not severe, patients are given Oral Rehydration Salts (ORS) to replace the loss of electrolytes, continued feeding as the patient experiences diarrhoea and administration of zinc for 10-14 days (Karambu *et al.*, 2013). In cases of severe diarrhoea, intravenous rehydration is done. Although sometimes administered, antidiarrheal drugs are not recommended, especially in acute diarrhoea. This is because they are not always effective. Some drugs that are administered to patients are ciprofloxacin and third-

generation fluoroquinolone have been recommended by the WHO as the first-line choice for patients with bloody diarrhoea (Gu *et al.*, 2012). Gu *et al.* (2012) states that the use of ciprofloxacin in paediatric patients is risky because it may cause damage to the growing cartilages, and so fluoroquinolones are generally used to treat shigellosis in children. These are prescribed to a patient when gastroenteritis is caused by a *Shigella* sp, and are normally given as a single dose, for two to five days (WGO 2012). WGO (2012) recommends that antimicrobial drugs should only be given to patients in cases of *Shigella*, *Salmonella* or *Campylobacter* infections, and in cases of moderate or severe traveller's diarrhoea.

Several studies in the Sahelian region (an ecoregion lying between the Sudianian savanna to the south and the Sahara desert to the north) have shown that multi-drug resistant organisms have been emerging over the last decade, with the general spread of resistance to amoxicillin, co-trimoxazole and chloramphenicol (Langendorf *et al.*, 2015). Langendorf *et al.* (2015) states that resistance to fluoroquinolones and extended-spectrum cephalosporins in Enterobacteriaceae have also been reported to a lower extent.

Another study conducted in Igembe District Hospital, Kenya showed that from the isolated bacteria (*Shigella* sp. *Salmonella* sp., ETEC, EPEC and EAEC), about 95% of them were resistant to amoxicillin, sulphinazazole and cotrimazole (Karambu *et al.*, 2013). Horizontal gene transfer and indiscriminating use of drugs has led to the *Shigella* sp. becoming resistant to commonly used antibiotics such as nalidixic acid, which is a widespread use first-line drug for shigellosis in many countries (Gu *et al.*,

2012). The study conducted by Gu et al. (2012) showed that resistance of *Shigella* sp. to nalidixic acid in Asia-Africa increased significantly, with a 12.1% resistance between the years 1998-2000 and a 64.5% resistance in the years 2007-2009. Finally, the study showed that resistance to ciprofloxacin were significantly less, with a 29.1% resistance in the years 2007-2009 (Gu *et al.*, 2012).

Patients that are vomiting can be given anti-emetic drugs. These drugs reduce fluid loss, thereby helping in oral rehydration therapy (Bannister, Gillespie and Jones, 2006). Examples of anti-emetic drugs given are cyclizine, promethazine and metoclopramide (Bannister, Gillespie and Jones, 2006). When managing GITI it is important, especially in infants to continue feeding. This reduces the recovery time needed as well as prevents excess weight loss.

2.7.2. Conventional treatment of Peptic Ulcer Disease (PUD)

Antibiotics are used in the treatment of PUD, however this may be problematic. According to Tille (2014) *H. pylori* can easily become resistant when metronidazole, clarithromycin, azithromycin, rifampin or ciprofloxacin are prescribed as a single agent. Currently, a triple-drug therapy is recommended. Oppong (2015) reports that this therapy includes metronidazole, a bismuth salt and either amoxicillin or tetracycline. A patient that has a metronidazole resistant strain may take omeprazole or lansoprazole together with amoxicillin or clarithromycin (Oppong *et al.*, 2015). Due to the increase in resistant strains to clarithromycin from 9% in 1998 over 20% in 2010, the triple-drug therapy has been based on the level of resistance (Oppong *et al.*, 2015).

2.8. Probiotics as Treatment for Diarrhoeal Symptoms GITI

As one of the major symptoms of GITI is diarrhoea, several studies have looked to probiotics as a form of treatment. Probiotics are defined as live microorganisms that when administered to a host may confer a health benefit (Madigan *et al.*, 2012). *Lactobacillus*, *Propionibacterium*, *Bacillus* and *Saccharomyces* are examples of strains that are normally used in production farm animals to inhibit digestive problems (Madigan *et al.*, 2012). Studies have been done to determine whether or not the use of probiotics can be used as an effective way to prevent and treat diarrhoea.

One study was conducted in India whereby *Lactobacillus casei*, *Saccharomyces boulardii* and *Bifidiobacterium lactis* were administered to children that obtained diarrhoea in the community and nosocomial diarrhoea (Guandalini, 2011). The study showed that the use of probiotics reduced the duration of diarrhoea when the probiotics were administered, especially when *S. boulardii* was taken in combination with metronidazole (Guandalini, 2011). Gunadalini (2011) states that, the use of probiotics as a treatment is less effective in treating bacterial diarrhoea than viral diarrhoea. Specifically, *Lactobacillus* GG was not found to be effective on diarrhoea of bacterial aetiology (Guandalini, 2011).

Different studies have demonstrated that probiotics can be effective in certain cases, resulting in varying results between and within individuals (Culligan, Hill and Sleator, 2009). Culligan *et al.* (2009) points out that the reason for this can be partly due to different modes of action of the probiotics as well as an increase in antibiotic resistance caused by an overuse and misuse of antibiotics. For example, a recent study showed

that *Bifidobacterium animalis* subsp. *lactis* failed to prevent common infections in hospitalized children (Vandenplas, 2016). With these being the case, medicinal plants have an important role to play to be able to reduce the cases of diarrhoea associated with GITI.

2.9. Medicinal Plants

Medicinal plants are plants that are used to treat infections, and their products are considered the most accessible and cheapest sources for the treatment and prevention of several diseases (Jaradat, Ayesh and Anderson, 2016). Throughout the world, these plants have been unique sources of medicines and constituted the most common human use of biodiversity (Olajuyigbe and Afolayan, 2012). They are said to make a major contribution to primary health care delivery as they are regarded as invaluable sources of pharmaceutical products (Olajuyigbe and Afolayan, 2012). There have been many reported cases of antimicrobial resistance and ineffective treatments with the conventional treatments currently being used to treat many ailments, including GITI such as PUD associated with *H. pylori*, of which treatment failure is up to 40% (Njume, Afolayan and Ndip, 2011). It is for this reason that medicinal plants have been considered as an alternative means to treat illnesses.

The study of medicinal plants and their uses began decades ago, and has since shown that incorporating them improves treatment. Africa is home to a remarkable diversity of medicinal plants and about 5400 plant species are used in traditional medicine in Africa (Van Wyk, 2015). Herbal medicine has a long history of usage and has become an important source of information for health care systems in both developed and

developing countries (Jaradat, Ayesh and Anderson, 2016). The use of traditional plants and plant-based medications are normally passed down from generation to generation, especially in Africa, and not usually documented (Olajuyigbe and Afolayan, 2012). This is why this research was aimed at investigating plants that could be used to treat symptoms associated with GITI in Namibia.

2.9.1. Traditional Uses Of Medicinal Plants

A research conducted in Kenya by Kimondo, *et al.* (2015) revealed that traditional medicine is preferred by the Ilkisonko Maasai community in Kenya, and only if the treatment fails, do they use conventional medicine, usually in combination with traditional medicine. One of the benefits of medicinal plants mentioned in this study, is their use as food as well as medicine (Kimondo *et al.*, 2015). In the study, it was also discovered that at least 54% of the members of the community used traditional medicine and indigenous foods once a month, which depended on the availability of meat, because the decoction made by boiling the plant would be mixed with bone soup or milk (Kimondo *et al.*, 2015). The mixture provides heat stable hydrophilic and lipophilic compounds that act as adaptogens and medicines. Herders take the decoction as an adaptogen when they have to walk for long distances in search of pasture (Kimondo *et al.*, 2015). Lastly, the study showed that stomach aches and constipation were one of the most common ailments treated by traditional medicine and that one of the most commonly used plants was *Ximenia* sp. which was also consumed as food (Kimondo *et al.*, 2015). Other plants that were shown to treat GIT disorders include *Rhus natalensis*, *Acacia mellifera*, *Salvadora persica*, *Pappea capensis*, *Solanum incanum*, *Urtica massaica*, and *Cyphostemma nodiglandulosum* (Kimondo *et al.*, 2015).

Research has shown that several other African countries are using medicinal plants to treat diarrhoea, indigestion, stomach cramps and other stomach related illnesses. These plants are *Acacia karroo*, *Ageratum conyzoides*, *Brucea antidysenterica*, and *Caltropis procera*. An ethnobotanical study conducted in the Eastern Cape, South Africa by Olajuyigbe and Afolayan (2012) confirms the use of *Acacia karroo* as an infusion and concoction of the leaves, bark and gum to treat diarrhoea, dysentery as well as haemorrhaging by members of the community. This study also showed that about 39% of the plants identified were used to treat diarrhoea as well as other symptoms of GITI, such as stomach pain, and examples of these plants are *Alepidea amatymbica*, *Cussonia spicata*, *Centella asiatica*, *Clausena anisate* and *Eucomis autumnalis* (Olajuyigbe and Afolayan, 2012). In Botswana, *Harpagophytum procumbens* is used to treat many ailments, as well as stomach ulcers diagnosed by a medical doctor (Mncwangi *et al.*, 2012).

In Namibia, information about medicinal plants used in the treatment of symptoms associated with GITI is anecdotal. Despite this being the case, several plants located in Namibia have been recorded as being used to treat diarrhoea. These include *Colophospermum mopane*, *Acanthosicyos horridus*, *Commiphora wildii*, *Harpagophytum procumbens*, *Sclerocarya birrea*, *Ximena americana* and *Xysmalobium undulatum*. *H. procumbens*' secondary tubers are used by the Topnaar people of Namibia, taken orally as a decoction or chewed to relieve stomach pain (Mncwangi *et al.*, 2012). With a lot more information being needed to be recorded, it is necessary to conduct more research to discover more plants and their activity against bacterial gastroenteritis and other GITI.

2.9.2. Phytochemistry

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Yadav and Agarwala, 2011). These biologically active compounds not only provide health benefits for humans but also protect plants from disease and damage and also contribute to the plant's colour, aroma and flavour (Kurmukov, 2013). These secondary metabolites have been known to inhabit different plant parts, including leaves, roots, barks and stems, and they are known to possess different properties (Kurmukov, 2013). **Table 4** shows the different classifications of phytochemicals and their biological function. Secondary metabolites include alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics and glycosides, and out of all of them, phenolics are the most numerous and structurally diverse plant phytoconstituents (Kurmukov, 2013).

Other plant secondary metabolites are lectins, proteins that have the ability to interact with carbohydrates or complex glycosidic structures (Van Buul and Brouns, 2014; Rodrigues *et al.*, 2016). Lectins possess antibacterial, anti-HIV, antitumour, anti-inflammatory, anti-proliferative functions and gastroprotective activity (Rodrigues *et al.*, 2016). A study by Rodrigues *et al.* (2016) revealed its gastroprotective property as purified lectins were able to protect the stomach from ethanol-induced damage. Rodrigues *et al.* (2016) suggests that this property, together with the antioxidant property that lectins possess are responsible for this capability.

Table 4: Bioactive Phytochemicals in Medicinal Plants

Classification	Main groups of compounds	Biological function
NSP (Non-starch polysaccharides)	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids
Antibacterial and antifungal	Terpenoids, alkaloids, phenolics, saponins, lectins	Inhibitors of micro-organisms, reduce the risk of fungal infections
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid, lectins	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols,(curcuminoids, flavonoids)	Inhibitors of tumour, inhibited development of lung cancer, anti-metastatic activity
Detoxifying agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Other	Alkaloids, terpenoids, volatile flavour compounds, biogenic amines	Neuropharmacologica agents,anti-oxidants, cancer chemoprevention

Source: Saxena *et al.* (2013)

CHAPTER 3: MATERIALS AND METHODS

3.1. Ethnobotanical survey

An ethnobotanical survey to obtain information about plants used to treat symptoms related to GITI was carried out in the Kavango and Ohangwena regions in northern Namibia. A collection permit was applied for at the Ministry of Environment and Tourism (**Appendix A**). The study was conducted in Rundu, in the Kavango region and Okamukwa in the Ohangwena region (**Figure 5**). In each study location, gatekeepers were used to obtain information about medicinal plants used to treat GITI and other people who know this information. In Rundu, the gatekeeper was a renowned traditional healer who is also a chairperson of the Traditional Healers Association in Namibia, registered with the Ministry of Health and Social Services (MOHSS) with vast experience in the traditional use of medicinal plants. Questionnaires were designed to obtain information about medicinal plants, their local names, the symptoms they treat as well as other uses (**Appendix B**). Information about how they are used to treat symptoms related to GITI was also obtained and included how the medicinal plants are prepared, how they are administered to the patient, as well as the part of the plant used. During the study, the names of the respondents were not revealed.

Eighteen plant species were collected from their natural habitats in the month of May 2016 in Rundu, Ncuncuni village and Okamukwa village and placed in paper bags and labelled. Purposive sampling method was used when collecting plants. Plant specimens for identification were put in plant presses and taken to the National Botanical Research Institute (NBRI).

3.4 *In vitro* antimicrobial activity

First, a susceptibility test was done by disk diffusion method. The extracts were tested in triplicates at a concentration of 100 mg/mL with gentamicin as a positive control and DCM:methanol as the negative control for the organic extracts and distilled water for the aqueous extracts. The bacteria used for the study were *E. coli*, *S. aureus*, *B. subtilis* and *S. sonnei*. They were inoculated on nutrient agar. The plates were incubated for 24 hours at 37°C. The data was then recorded by measuring the zone of inhibition. Dilutions were done for plant extracts that had the highest inhibitory effect against each bacteria with concentrations ranging from 0.01 mg/mL to 100 mg/mL. The zones of inhibition were calculated in millimetres and recorded. This was done so as to determine the minimum inhibitory concentration (MIC).

3.5. Fractionation of samples

Selected plant extracts were selected for fractionation by vacuum liquid chromatography (VLC). The following solvents were used with increase in polarity: 10% acetone in dichloromethane (DCM), 50% acetone in DCM, 20% methanol in DCM, acetone, ethyl acetate and methanol. The samples were then concentrated using a rotary evaporator and completely dried in a fume hood. Dimethyl sulfoxide (DMSO) was used to make stock solutions of the extracts at a concentration of 100 mg/mL. DMSO was used as a negative control.

3.6. Data analysis

Independent Samples Kruskal Wallis test was used to determine whether there was a significant difference between the tested extracts, followed by a Tukey test as a post-hoc test for pairwise comparisons and ranking of samples for groups that had a significant difference with the independent samples Kruskal Wallis test.

CHAPTER 4: RESULTS

4.1. Ethnobotanical survey

From the survey, 10 interviews were conducted and information about the respondent, their knowledge about medicinal plants and how they were being used as well as the habitat of the plants were obtained. From the study, all the respondents were over the age of 60, for which one was male and the rest were female. Of these respondents, one was a male traditional healer who has been practicing for 46 years and claimed to obtain his indigenous knowledge from spirits. The other respondents were a headwoman from Rundu, Kaisosi and the rest, elderly ladies from the Ohangwena region who made their living by farming.

The information they have about medicinal plants were passed down to them through generations. Of all the plants brought up by the respondents, the most mentioned ones were *Aloe* sp. Baker and *H. procumbens*. When using plants for medicinal purposes, the study showed that the most plant part used were roots (**Figure 6**), and the least plant part used were the whole plant and the bark, and the most frequently plant family used were from Leguminosae and Anacardiaceae. (**Table 5**). **Picture 1 – 6** show some of the plants used for this study in their natural habitat.

Table 5: Medicinal plants used to treat GIT symptoms in the Kavango and Ohangwena regions of Namibia

Local name	Family	Scientific name	Region	Disease treated	Plant part used, preparation and mode of administration
Ekakata/ Likakata	Pedaliaceae	<i>Harpagophytum procumbens</i> (Burch.) DC ex Meisn.	Kavango, Ncuncuni village	Treat stomach cramps, reduces malaria	An infusion is done with the roots in cold water and left overnight. The water is taken orally
Okalupulupu	Asteraceae	<i>Dicoma tomentosa</i> Cass.	Ohangwena, Okamukwa village	Fevers, constipation, best used when one is vomiting	The whole plant is infused in water and it is taken orally
Mutakatabolo	Apiaceae	<i>Steganotaenia araliacea</i> Hochst.	Kavango, Ncuncuni village	Infertility, malaria and stomach pains, bleeding in the stomach	The roots are infused in water overnight and the water taken orally
Sihopan	Aloaceae	<i>Aloe sp. Baker</i>	Kavango, Rundu	Stomach pain, wounds on the skin, malaria, allergies, treats swollen liver and bile, reduces fever	The leaves are put in water and left to boil. The water is taken orally, 3 times a day
Moringa	Moringaceae	<i>Moringa oleifera</i>	Kavango, Rundu	Treats stomach pain, reduces malaria.	The leaves are dried in the shade, crushed into a powder and put in food. Treatment takes 2 – 3 days
Ndonga	Convolvulaceae	<i>Ipomoea hochstetteri</i> House	Kavango, Rundu	Cleans out the blood in the stomach, treats stomach pain and treats symptoms related to malaria	Make an infusion with water then drink the water

Omuhonono	Combretaceae	<i>Terminalia sericea</i> Burch. Ex DC.	Kavango, Ncuncuni village	Treats stomach pain (roots), swollen legs (leaves), barks are edible	The roots are crushed to soften, put in water overnight, then boil for a few minutes, then put in the anus to release impurities from the stomach
Mulia	Apocynaceae	<i>Diplorhynchus condylocarpon</i> (Mull. Arg.) Pichon	Kavango, Ncuncuni village	Stomach problems	Infusion with cold water, which is drunk
Mukumati	Anacardiaceae	<i>Searsia tenuinervis</i> (Engl.) Moffett	Kavango, Ncuncuni village	Stomach pain	The roots are put in cold water in a bottle, left overnight then drunk
Onzupeke	Olacaceae	<i>Ximenia caffra</i> Sond. Var <i>caffra</i>	Kavango, Ncuncuni village	Stomach pain and diarrhoea	Infusions of the roots are used
Muchansi/Kabula bula	Chrysobalanaceae	<i>Parinari capensis</i> Harv	Kavango, Ncuncuni village	Treats stomach pain, sores in the stomach, treats infertility	The roots are ground into a powder and put in food
Musu	Leguminosae	<i>Acacia erioloba</i> E. Mey	Kavango, Rundu	Stomach pains	Leaves are mashed with <i>G. senegalensis</i> in water and then drink the water.
Mungorwe	Celastraceae	<i>Gymnosporia senegalensis</i> (Lam.) Loes	Kavango, Rundu	Stomach pain	Leaves are mashed with <i>A. erioloba</i> in water and then drink the water.
Okapata	Leguminosae	<i>Tephrosia burchellii</i> Burt Davy	Ohangwena, Okamukwa	Watery, bloody diarrhoea.	The roots are ground and mixed with water and taken orally. The roots can be mixed with mahangu flour to make bread. They can be ground when fresh or dry

Okapata	Molluginaceae	<i>Mollugo cerviana</i> (L.) <i>Ser. ex DC</i>	Ohangwena, Okamukwa	Treats diarrhoea in both humans and cattle	The whole plant is dried and made into a powder, mixed with water and taken orally
Marula	Anacardiaceae	<i>Sclerocarya birrea</i> (A. Rich) <i>Hochst subsp. Caffra</i> (Sond.) <i>Kokwaro</i>	Ohangwena, Okamukwa	Diarrhoea, stomach pain	A decoction is made with the leaves using water and this is drunk
Omukwiyu	Moraceae	<i>Ficus sycomorus</i> <i>L. subsp. gnaphalocarpa</i>	Ohangwena, Okamukwa	Diarrhoea	The bark is pound, make a decoction with water, which is taken orally
Ondhingulula	Asclepiadaceae	<i>Marsdenia sylvestris</i> (Retz.) <i>P.I Forst</i>	Ohangwena, Okamukwa	Stomach problems	Pound roots and put them in cold water. This can be taken orally, or inserted into the anus

Note: Local name of plants in Kavango are in Silozi as the traditional healer is from there, except for *G. senegalensis* and *A. erioloba*, which are in Rukwangari. Local name of plants located in Ohangwena region are in Oshiwambo

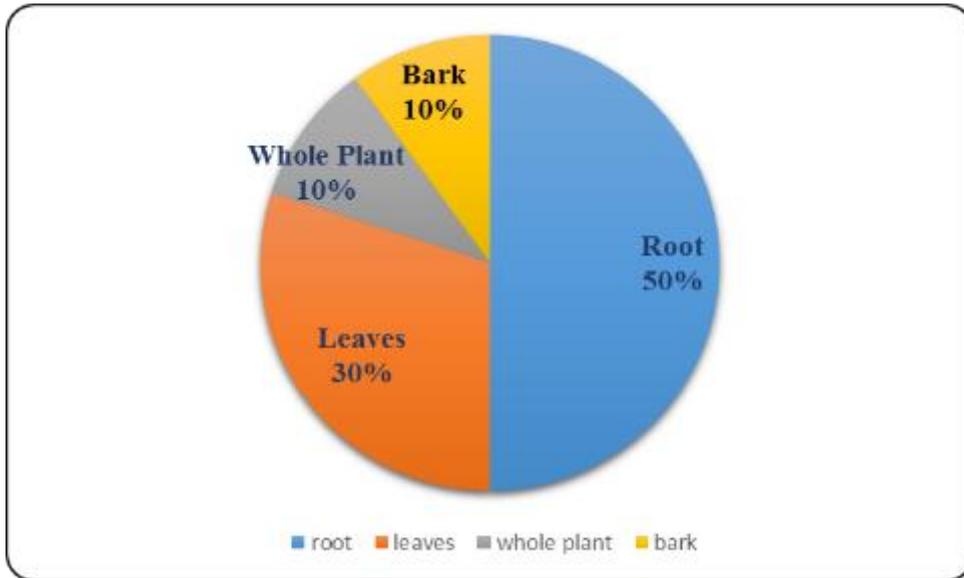


Figure 6: Percentage of medicinal plant parts used to treat GITI.



Terminalia sericea



Diplorhynchus condylocarpon

Picture 1: Photo of *T. sericea* and *D. condylocarpon* in their natural habitat in Ncuncuni, Kavango region.



Parinari capensis



Acacia erioloba

Picture 2: Photo of *P. capensis* and *A. erioloba* in their natural habitat in the Kavango region.



Steganotaenia araliacea Hochst



Searsia tenuinervis

Picture 3: Photo of *S. araliacea* and *S. tenuinervis* in their natural habitat at Ncuncuni village, Kavango region.



Ipomoea hochstetteri



Mollugo cerviana

Picture 4: Photo of *I. hochstetteri* in Rundu, Kavango region and *M. cerviana* in Okamukwa village, Ohangwena region their natural habitats.



Harpagophytum procumbens



Moringa oleifera

Picture 5: Photo of *H. procumbens* in Ncuncuni village and *M. oleifera* in Rundu in the Kavango region.



Aloe sp.



Ximenia caffra

Picture 6: Photo of *Aloe sp.* and *X. caffra* in their natural habitat in Ncuncuni village, Kavango region.

4.2. Antimicrobial assay

4.2.1. Antimicrobial assay of crude extracts

Twenty extracts were tested on *E. coli*, *B. subtilis*, *S. sonnei* and *S. aureus* at a concentration of 100 mg/mL (**Table 6**). When tested against *B. subtilis*, majority of the aqueous extracts, (80%), had activity against the organism, with *X. caffra* having the

highest zone of inhibition, with an average of 11 mm (**Figure 7**). Sixty percent of the aqueous plant extracts tested on *S. aureus* had activity, with *T. sericea* root extracts having the highest average zone of inhibition of 8 mm (**Figure 8**). *S. sonnei* was the organism whose growth was the least inhibited when tested against all the extracts. Of these extracts, only 20% of them showed antibacterial activity, with *I. hochstetteri* having the highest antibacterial effect with an average zone of inhibition of 6 mm as shown in **Figure 9**. Of these twenty extracts, 45% of the aqueous extracts had activity against *E.coli*, with *H. procumbens* having the most activity, with a mean zone inhibition of 12 mm (**Figure 10**).

At 100 mg/mL organic crude extracts from *T. sericea* were seen to have the highest activity in three of the microorganisms, *S. aureus*, *E. coli* and *S. sonnei*. *T. sericea* bark extract had an average zone of inhibition of 13 mm on *S. sonnei* (**Figure 9**). *T. sericea* root extracts had the highest activity on both *S. aureus* and *E. coli*, with zones of inhibition of 15 mm and 14 mm respectively (**Figure 8 & 10**). About half of the plant extracts had activity against *B. subtilis*, *E. coli* and *S. sonnei*, while 70% of all the extracts were seen to have activity against *S. aureus*

Table 6: Antimicrobial activity of different treatments

Treatment	<i>B. subtilis</i>		<i>S. aureus</i>		<i>S. sonnei</i>		<i>E. coli</i>	
	Aqueous	Organic	Aqueous	Organic	Aqueous	Organic	Aqueous	Organic
<i>A. erioloba</i>	+	+	+	+	-	+	-	+
<i>Aloe sp.</i>	-	+	-	+	-	+	-	+
<i>D. condylocarpon</i>	-	+	+	+	-	-	+	+
<i>D. tomentosa</i>	+	-	-	-	-	+	-	-
<i>F. sycomorus</i>	+	-	+	-	-	-	-	-
<i>G. senegalensis</i>	+	+	-	+	-	-	+	+
<i>H. procumbens</i>	+	-	-	-	-	-	+	-
<i>I. hochstetteri</i>	+	+	+	+	+	-	+	-
<i>M. cerviana</i>	-	+	+	+	-	+	+	+
<i>M. oleifera</i>	-	+	-	-	-	-	-	-
<i>M. sylvestris</i>	+	+	+	+	-	-	-	-

<i>P. capensis</i>	+	+	+	+	+	+	+	+
<i>S. araliacea</i>	+	-	-	-	-	+	-	+
<i>S. birrea</i>	+	-	-	-	-	-	-	+
<i>S. tenuinervis</i>	+	-	+	+	-	-	+	+
<i>T. burchellii</i>	+	+	+	+	+	-	-	+
<i>T. sericea (rt)</i>	+	-	+	+	-	+	+	+
<i>T. sericea (brk)</i>	+	-	-	+	-	+	-	-
<i>T. sericea (lv)</i>	+	-	+	+	-	+	-	-
<i>X. caffra</i>	+	+	+	+	+	+	+	+

Key: + Antimicrobial activity

- No antimicrobial activity

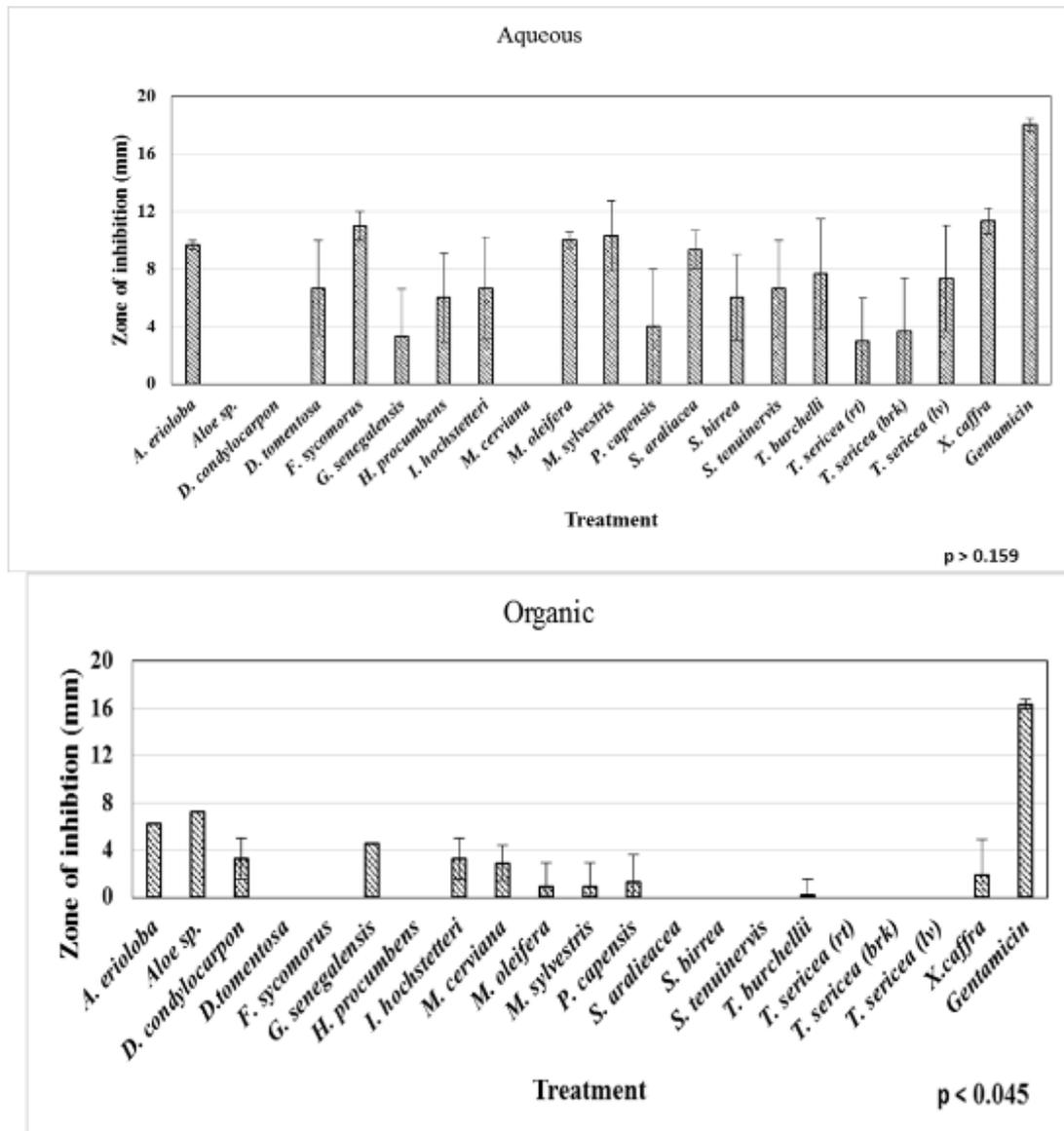


Figure 7: Antimicrobial activity of crude extracts (aqueous and organic) against *B. subtilis* at concentration of 100 mg/mL. Means \pm S E of 3 replicates are represented. No inhibition was observed with distilled water as a control.

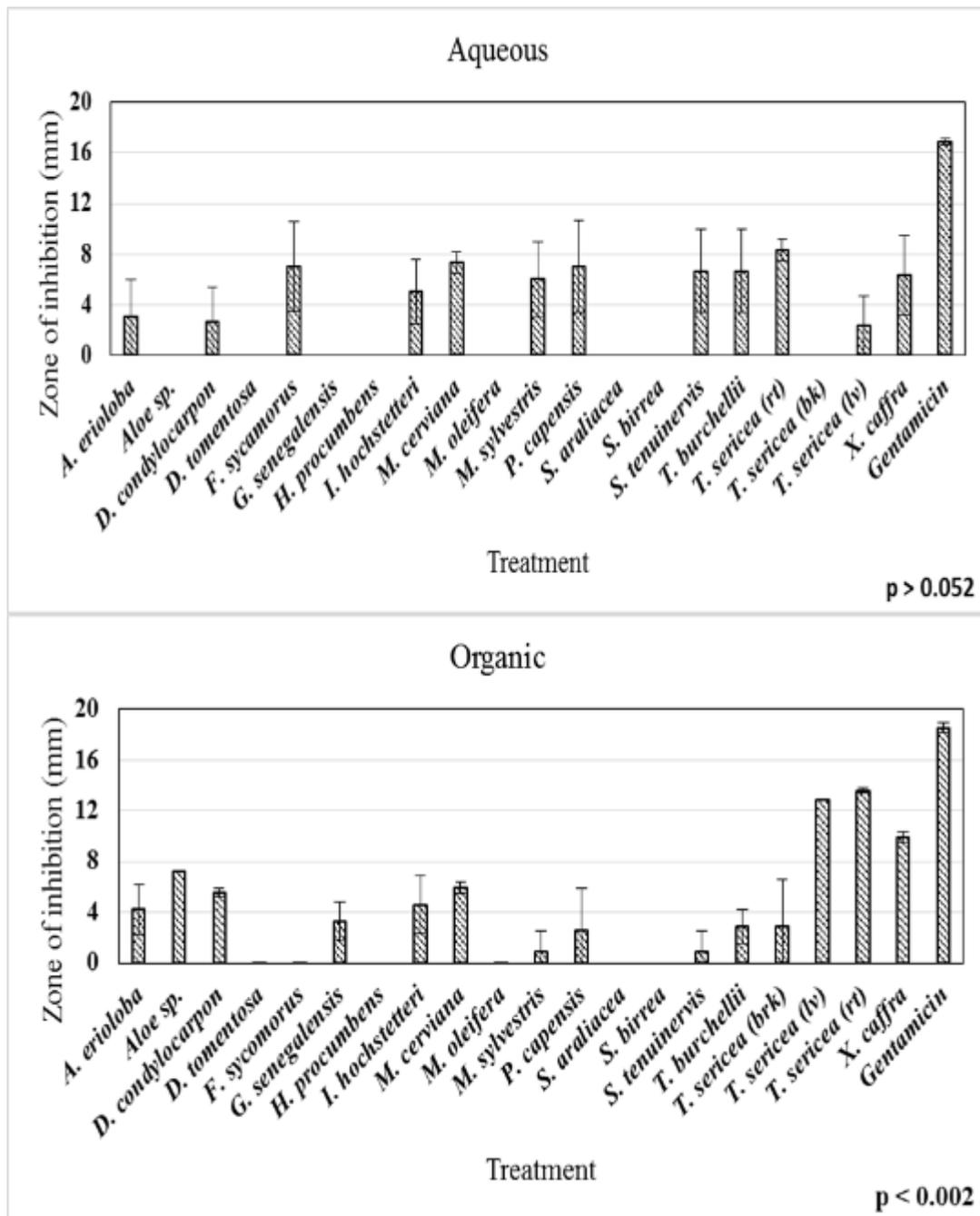


Figure 8: Antimicrobial activity of crude aqueous and organic extracts against *S. aureus*. Mean \pm SE of 3 replicates are represented. No inhibition was observed with distilled water as a control.

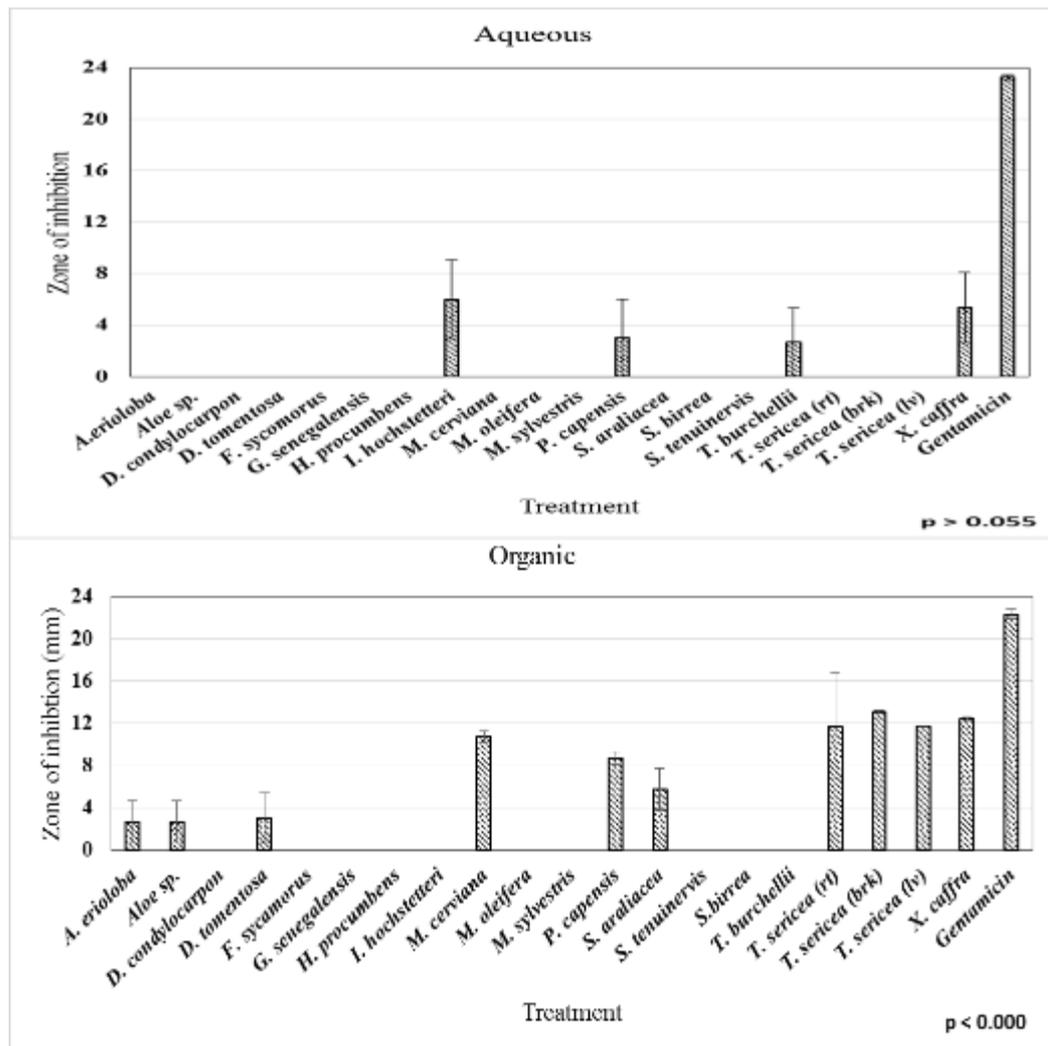


Figure 9: Antimicrobial activity of crude aqueous and organic extracts of medicinal plants against *S. sonnei*. Means \pm SE of 3 replicates are represented. No inhibition was observed with distilled water as a control.

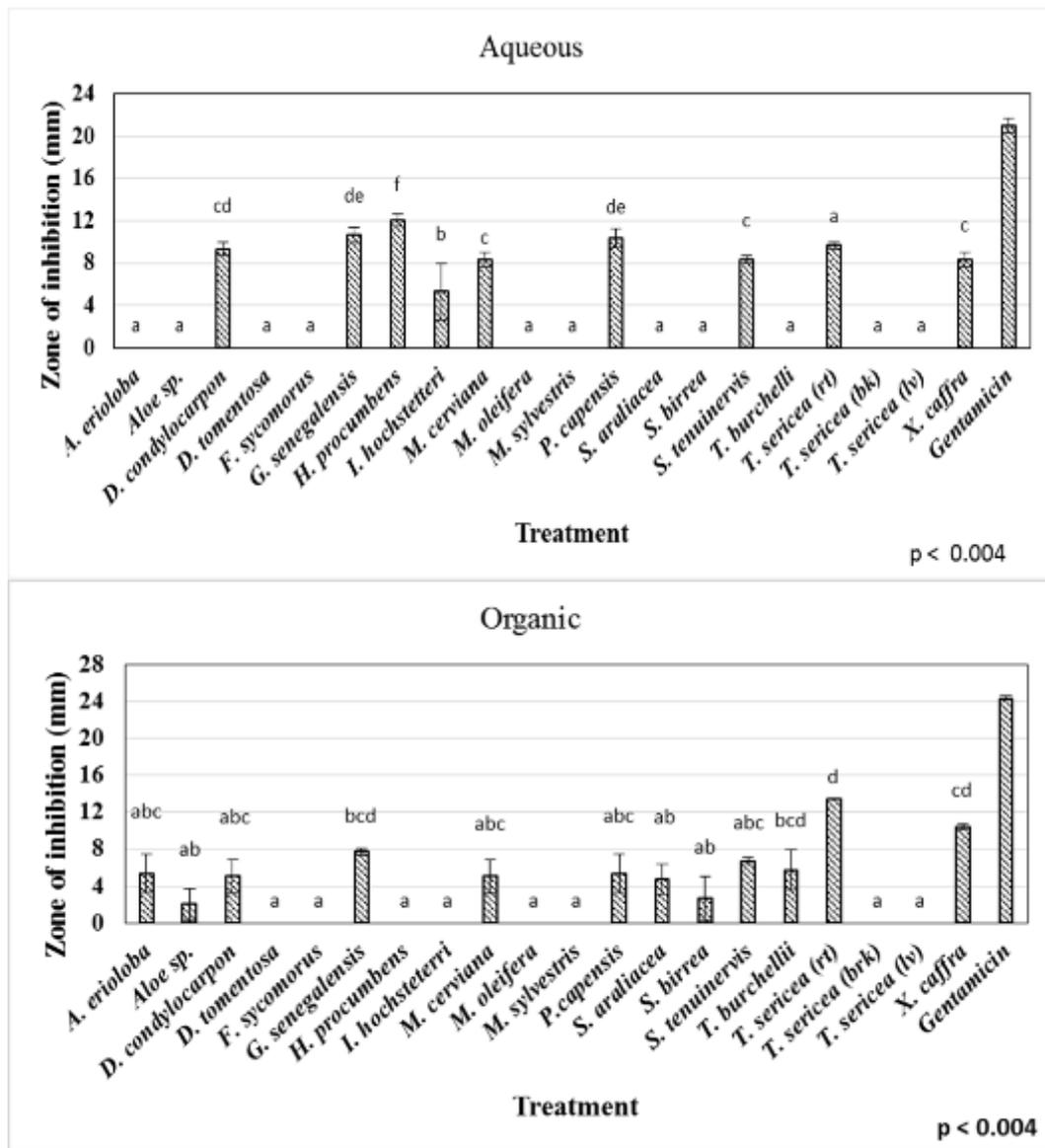


Figure 10: Antimicrobial activity of crude aqueous and organic extracts against *E. coli*. Mean \pm SE of 3 replicates are represented. No inhibition was observed with distilled water as a control. Extracts with the same letters are not significantly different according to Tukey's test. Kruskal-Wallis test p-value shown.

P. capensis, *G. senegalensis* and *H. procumbens* aqueous extracts were selected to test at concentrations ranging from 10 mg/mL to 0.01 mg/mL on *E. coli*, and *M. oleifera*, *F. sycomorus* and *X. caffra* on *B. subtilis* (**Figure 11 and 12**). No activity was seen for *G. senegalensis* and *F. sycomorus* (**Table 7**), while *H. procumbens* remained active against *E. coli* even at the lowest concentration (0.01 mg/mL) as shown in **Figure 11**. Errors in accuracy and precision were experienced during the study as some data were

seen to have large standard errors. These data are shown in **Table 7** and **Table 8**. For example, the antimicrobial of *P. capensis* against *E. coli* showed a zone of inhibition of $2\pm 2\text{mm}$ (**Table 7**), showing that the measurements taken had large differences, leading to a large standard error.

Table 7: Antimicrobial activity of selected aqueous plant extracts on *B. subtilis* and *E. coli* at different concentrations

Bacteria	Treatment	Part	Average zone of inhibition (mm)±SE at different concentrations			
			10 mg/mL	1 mg/mL	0.1 mg/mL	0.01 mg/mL
<i>B. subtilis</i>	<i>X. caffra</i>	RT	8 ± 1	5 ± 3	8±0.3	3±0
	<i>F. sycomorus</i>	BK	NA	NA	NA	NA
	<i>M. oleifera</i>	LV	3 ±3	NA	5±0.	5±2
<i>E. coli</i>	<i>H. procumbens</i>	RT	5±3	6±3	5±2	9±1
	<i>P. capensis</i>	RT	9±1	2±2	NA	NA
	<i>G. senegalensis</i>	LV	NA	NA	NA	NA

Key: LV=Leaves; BK=bark; RT=Roots; WP=Whole plant; NA=No activity; SE = Standard error

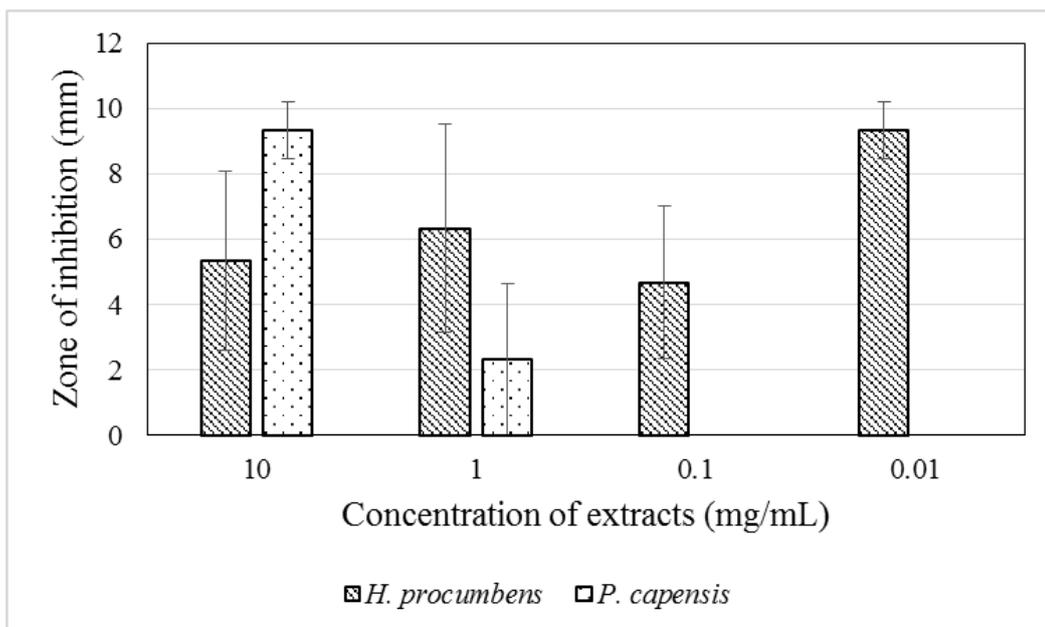


Figure 11: Antimicrobial activity of aqueous extracts of *H. procumbens* and *P. capensis* on *E. coli* at different concentrations. Mean \pm SE of 3 replicates are represented.

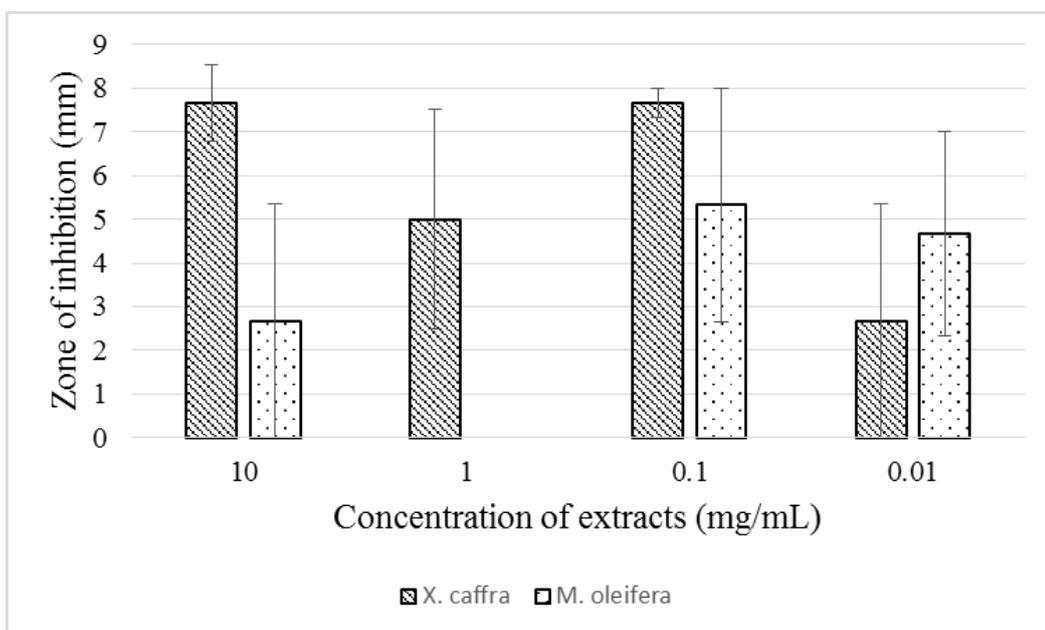


Figure 12: Antimicrobial activity of aqueous extracts of *X. caffra* and *M. oleifera* on *B. subtilis* at different concentrations. Mean \pm SE of 3 replicates are represented.

The organic extracts for *A. erioloba*, *Aloe sp.*, *X. caffra*, *T. sericea* roots, leaves and bark, and *T. burchellii* were selected to be tested at concentrations ranging from 10 mg/mL to 0.01 mg/mL. *A. erioloba* and *Aloe sp.* were tested on *B. subtilis* and did not have any activity at these concentrations (**Table 8**). The extracts from *T. sericea* roots and leaves and *X. caffra* were tested on *S. aureus* at concentrations ranging from 10 mg/mL to 0.01 mg/mL (**Figure 13**). **Figure 14 & 15** further show the antimicrobial activity of organic plant extracts at different concentrations against *S. sonnei* and *E. coli*. **Figure 16** compares the antimicrobial activity of *T. sericea* root and *X. caffra* extracts against *S. sonnei*, *S. aureus* and *E. coli*. In this comparison, it can be seen that *T. sericea* had a weaker antimicrobial activity against *E. coli*, as compared to the other bacteria, whereas *X. caffra* had antimicrobial activity against all bacteria up to 1 mg/mL

Table 8: Antimicrobial activity of selected organic plant extracts on *B. subtilis*, *S. aureus*, *S. sonnei* and *E. coli* at different concentrations

Bacteria	Treatment	Part	Average zone of inhibition (mm) \pm SE at different concentrations			
			10 mg/mL	1 mg/mL	0.1 mg/mL	0.01 mg/mL
<i>B. subtilis</i>	<i>A. erioloba</i>	LV	NA	NA	NA	NA
	<i>Aloe sp.</i>	LV	NA	NA	NA	NA
<i>S. aureus</i>	<i>T. sericea</i>	LV	8 \pm 4	5 \pm 3	5 \pm 3	NA
	<i>T. sericea</i>	RT	18 \pm 0.3	10 \pm 1	3 \pm 3	NA
	<i>X. caffra</i>	RT	12 \pm 0.9	3 \pm 3	NA	NA
<i>E. coli</i>	<i>T. burchellii</i>	RT	NA	NA	NA	NA
	<i>T. sericea</i>	RT	NA	NA	NA	NA
	<i>X. caffra</i>	RT	13 \pm 1	10 \pm 0.3	NA	NA
<i>S. sonnei</i>	<i>T. sericea</i>	BK	8 \pm 4	NA	NA	NA
	<i>T. sericea</i>	LV	NA	NA	NA	NA
	<i>T. sericea</i>	RT	11 \pm 1	6 \pm 3	NA	NA
	<i>X. caffra</i>	RT	12 \pm 1	3 \pm 3	NA	NA

Key: LV=Leaves; BK=bark; RT=Roots; WP=Whole plant, NA= No activity; SE=Standard Error

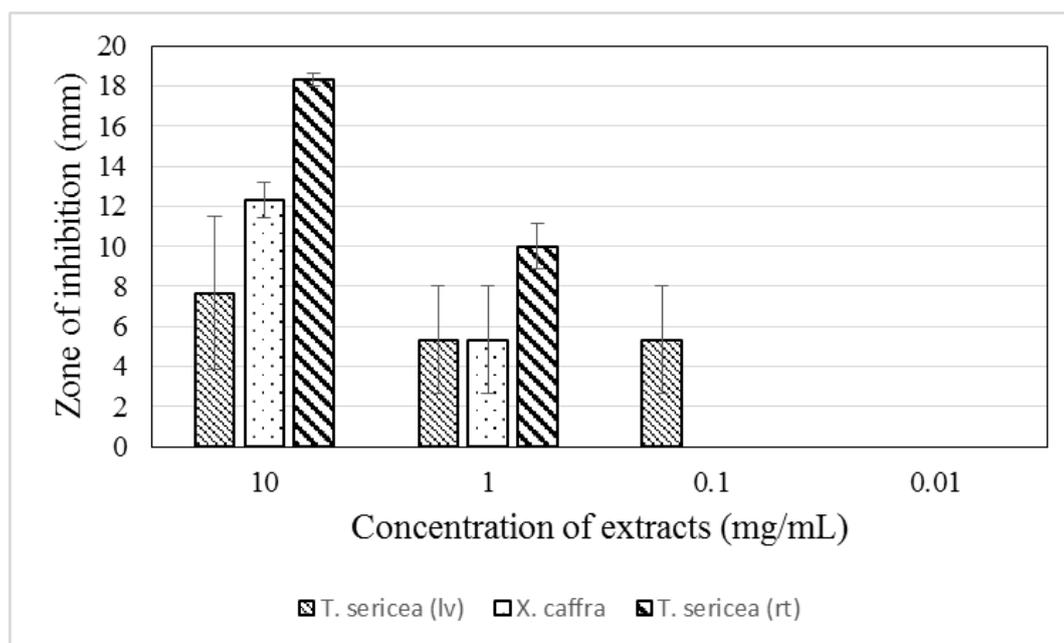


Figure 13: Antimicrobial activity of selected organic extracts on *S. aureus* at different concentrations. Mean \pm SE of 3 replicates are represented.

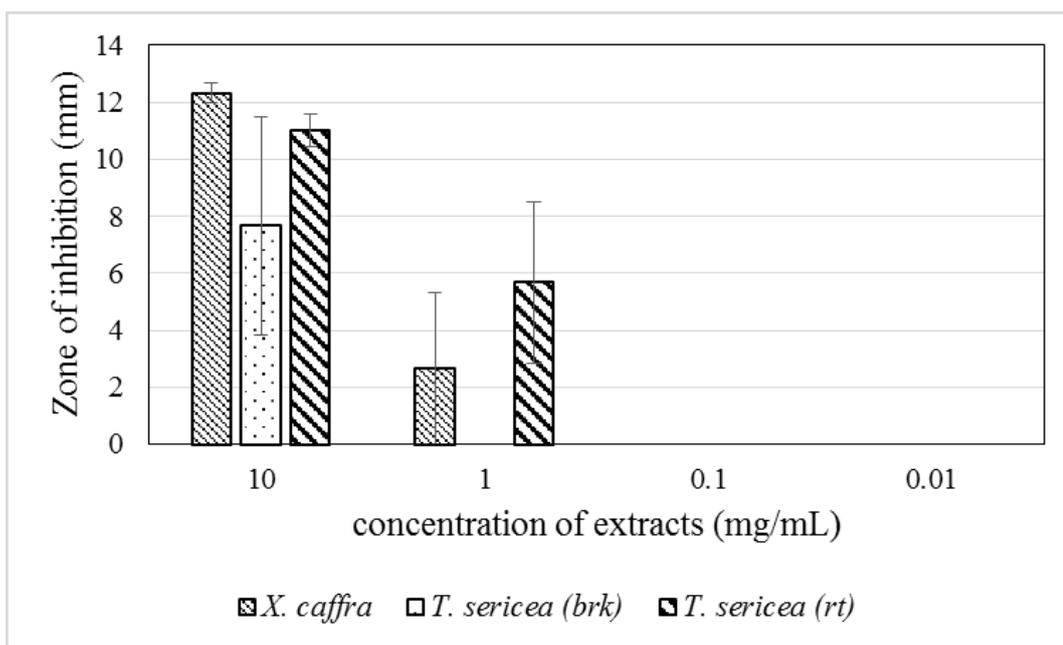


Figure 14: Antimicrobial activity of selected plant extracts on *S. sonnei* at different concentrations. Mean \pm SE of 3 replicates are represented.

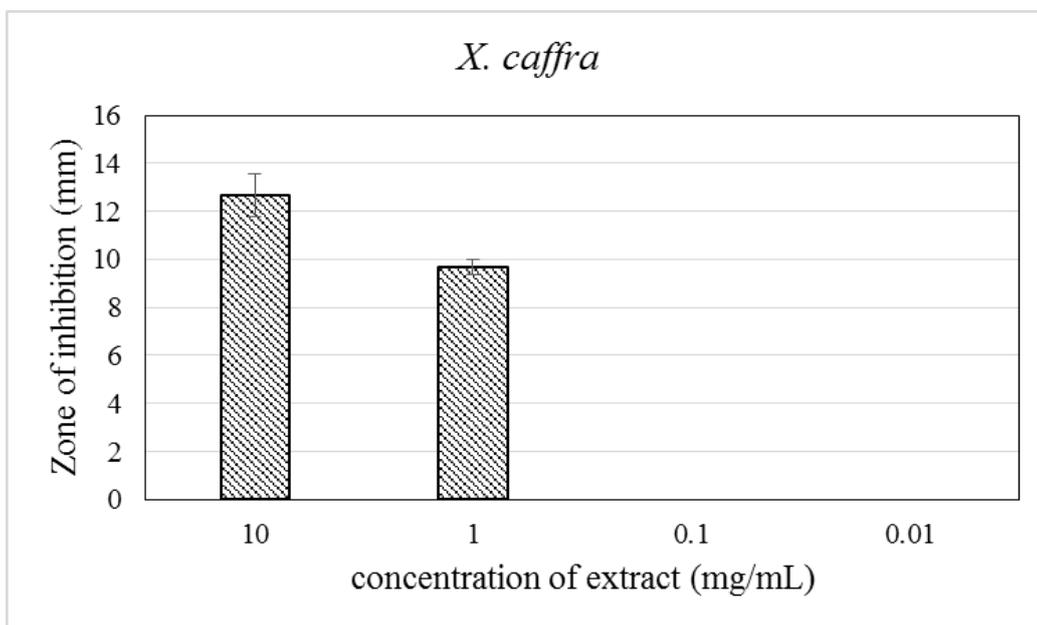


Figure 15: Antimicrobial activity of the organic extract of *X. caffra* on *E. coli* at different concentrations.

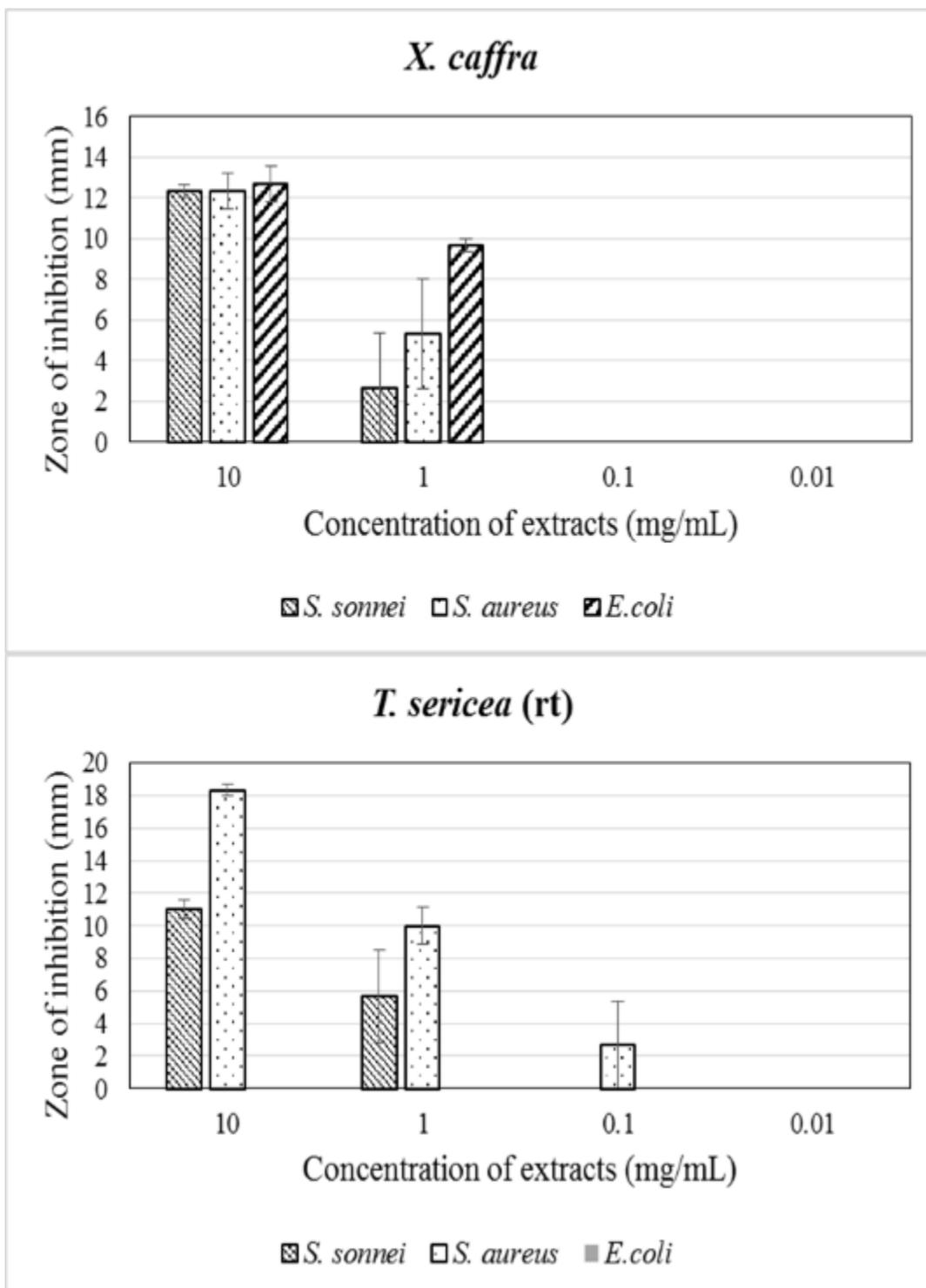


Figure 16: Antimicrobial activity of selected organic plant extracts on *S. sonnei*, *S. aureus* and *E. coli* at different concentrations

4.2.2 Antimicrobial activity of fractionated samples

As *X. caffra* and *T. sericea* root extracts had the most activity against *E. coli*, *S. aureus* and *S. sonnei* they were chosen for fractionation by VLC so as to determine which fraction produced the most activity. As seen in **Figure 17**, the active principal in Ethyl acetate fraction of *T. sericea* root extract had the most activity against *E. coli*. Against *S. sonnei* and *S. aureus*, extracts from 10% acetone in DCM and 20% methanol in DCM fractions had the most activity, respectively. Against *E. coli*, the active principal of *T. sericea* from ethyl acetate fraction and that of the methanol fraction from *X. caffra* had a minimum inhibitory concentration (MIC) of 100 mg/mL. The active principal from 10% acetone in DCM fractions produced from *T. sericea* root extract and *X. caffra* both showed that the MIC against *S. sonnei* was 0.01 mg/mL (**Table 10; Figure 19; Figure 18**). *T. sericea* extract produced from 20% methanol in DCM extract had an MIC of 0.01 mg/mL when tested against *S. aureus*, while the *X. caffra* extract from 100% methanol fraction had an MIC of 0.1 mg/mL (**Figure 18 & 19**).

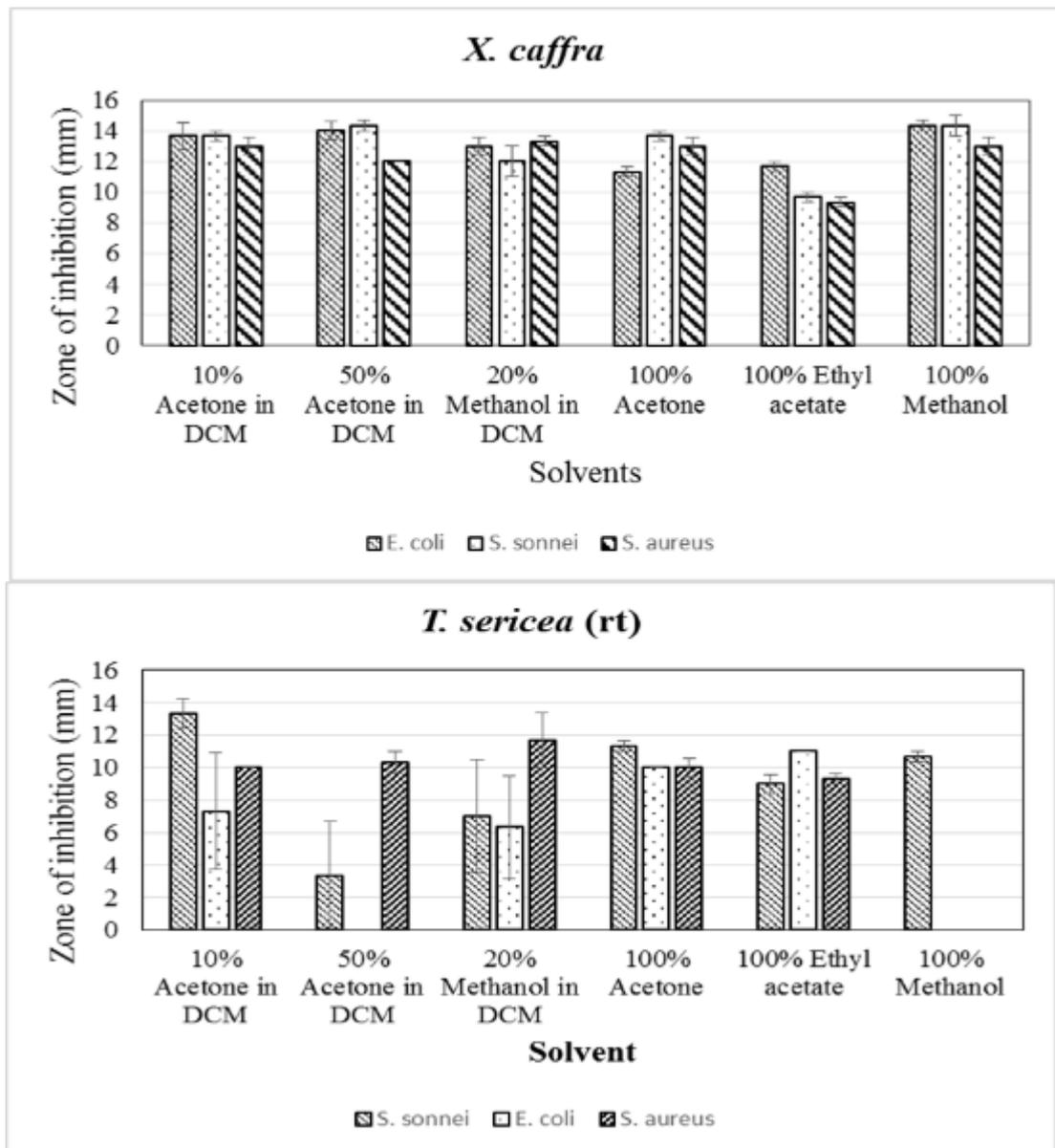


Figure 17: Antimicrobial activity of fractionated samples at 100 mg/mL of *X. caffra* and *T. sericea*. Mean \pm SE of 3 replicates represented.



A



B



Picture 7: Antimicrobial activity of A) *T. sericea* root fractions and B) *X. caffra* fractions on *S. sonnei* at 100 mg/mL.



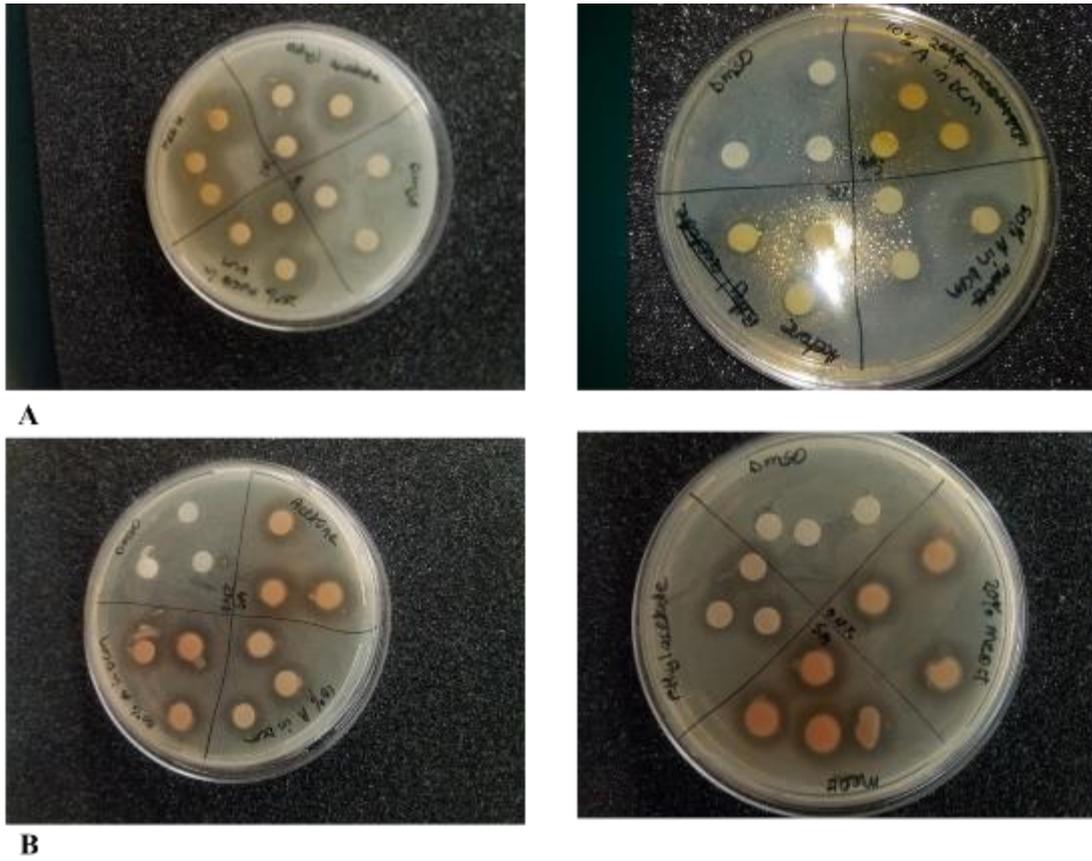
A



B



Picture 8: Antimicrobial activity of A) *T. sericea* root fractions and B) *X. caffra* fractions on *E. coli* at 100 mg/mL.



Picture 9: Antimicrobial activity of A) *T. sericea* root fractions and B) *X. caffra* fractions on *S. aureus* at 100 mg/mL.

In **Picture 7, 8** and **9**, the zones of inhibition, showing the antimicrobial activity of selected fractions are shown. Activity against *E. coli*, *S. aureus* and *S. sonnei* are shown. **Picture 10** and **11** show the fractions produced from vacuum liquid chromatography.

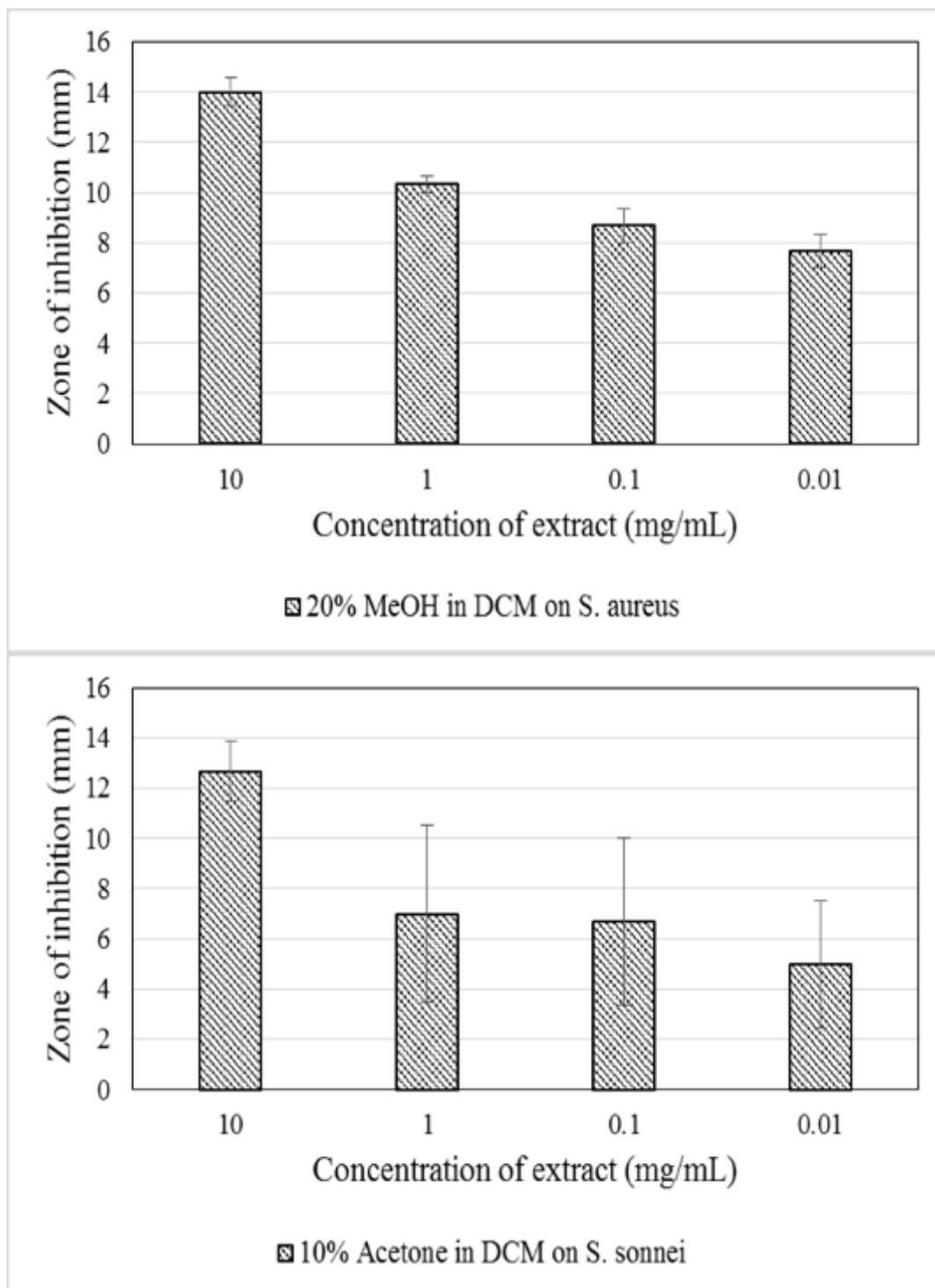


Figure 18: Antimicrobial activity of *T. sericea* (root) 20% methanol in DCM on *S. aureus* and *T. sericea* (root) 10% Acetone in DCM on *S. sonnei* at different concentrations. Mean \pm SE in 3 replicates is represented.

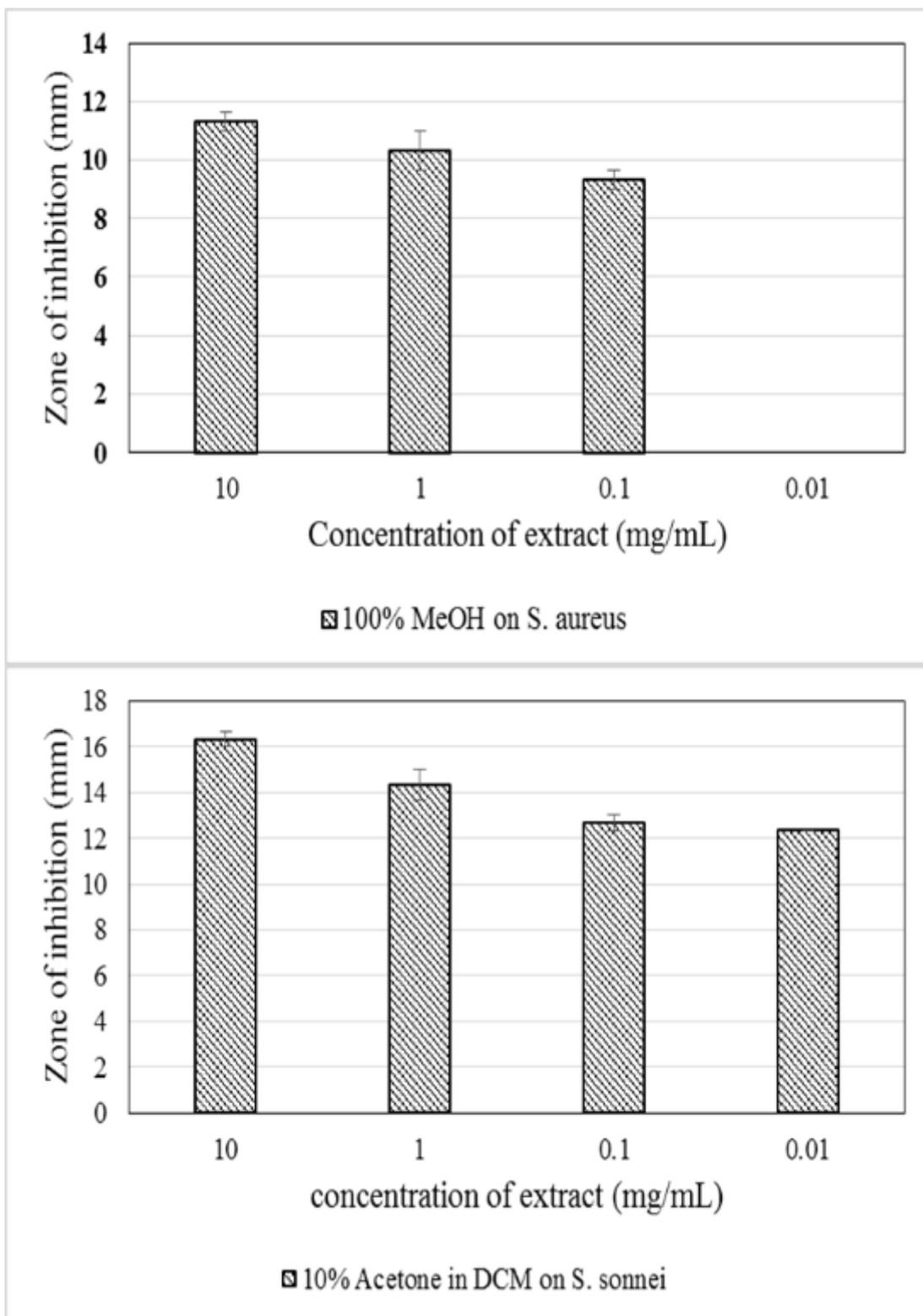


Figure 19: *X. caffra* antimicrobial activity with 100% methanol solvent on *S. aureus* and 10% acetone in DCM on *S. sonnei*. Mean \pm SE of 3 replicates represented.



Picture 10: Fractionated samples from *X. caffra* crude extract.



Picture 11: Fractionated samples from *T. sericea* root crude extracts.

Table 9: Minimum inhibitory concentration (MIC) of selected organic plant extracts

Bacteria	Plant extract	Part	MIC(mg/mL)
<i>B. subtilis</i>	<i>A. erioloba</i>	LV	100
	<i>Aloe sp.</i>	LV	100
<i>S. aureus</i>	<i>X. caffra</i>	RT	1
	<i>T. sericea</i>	RT	0.1
	<i>T. sericea</i>	LV	0.1
<i>E. coli</i>	<i>X. caffra</i>	RT	1
	<i>T. sericea</i>	RT	100
	<i>T. burchellii</i>	RT	100
<i>S. sonnei</i>	<i>X. caffra</i>	RT	1
	<i>T. sericea</i>	BK	10
	<i>T. sericea</i>	LV	100
	<i>T. sericea</i>	RT	1

Table 10: Minimum inhibitory concentration (MIC) of selected active fractions

Bacteria	Fraction	Plant extract	Part	MIC (mg/mL)
<i>E. coli</i>	100% Ethyl acetate	<i>T. sericea</i>	RT	100
	100 Methanol	<i>X. caffra</i>	RT	100
<i>S. aureus</i>	20% Methanol in DCM	<i>T. sericea</i>	RT	0.01
	100% methanol	<i>X. caffra</i>	RT	0.1
<i>S. sonnei</i>	10% Acetone in DCM	<i>T. sericea</i>	RT	0.01
	10% Acetone in DCM	<i>X. caffra</i>	RT	0.01

CHAPTER 5: DISCUSSION

5.1. Ethnobotanical survey

The ethnobotanical survey showed that there are plants used in a traditional setting to treat symptoms related to GITI in Namibia. As seen in the results, eighteen plants were mentioned. Majority of the plant parts used to treat these symptoms are roots, followed by leaves, the whole plant and the bark. This result is in accord with a study conducted in the Oshikoto region, Namibia, with the least plant part used for medicinal purposes after the four mentioned being tubers, seeds, fruits, pods and stems (Cheikhoussef *et al.*, 2011). This study discusses the medicinal plants in the Oshikoto region of Namibia and their uses. Also mentioned in the study by Cheikhoussef *et al.* (2011) is the age of the respondents in the survey, which were above the age of 60, as is the case in this study. This shows that indigenous knowledge is held by the elderly in the community.

Many studies have reported the different traditional uses of the plants stated in the results. Some of these uses are listed in **Table 10**, the other plants traditional uses are explained in more detail below as information on their uses have been significantly mentioned. Firstly, number of reports have documented the traditional use of *A. erioloba*, a tree commonly known as camel thorn. For instance the wood ash and bark are applied topically to treat skin disease (Mabona and Van Vuuren, 2013; Chinsembu, Hjarunguru and Mbangu, 2015), the leaves, roots, thorns and bark as treatment for persistent coughing, abdominal pain, headache, diarrhoea, fever and coughing (Du Preez, 2012). These stated uses of *A. erioloba* confirm the reported medicinal uses of the leaves in this study.

The Ethnobotanical survey informed that the leaves of *Aloe* sp. are used to treat diarrhoea. A number of *Aloe* sp. are being used to treat different ailments, including symptoms related to GITI. In Angola, a study revealed that *A. littoralis* leaves are used in the treatment of diarrhoea, backache and burns (Urso *et al.*, 2016). Another species, *A. vera* is used to treat constipation, dysentery, indigestion, intestinal worms, peptic ulcers, stomach disorders, stomach ache, hepatic stimulant, and haemorrhoids (Rokaya *et al.*, 2014). *A. ferox* leaves have also been reported as being used for medicinal purposes including its role as an emetic (Ndhlala *et al.*, 2013).

F. sycomorosa, a medicinal plant from the family Moraceae, has medicinal purposes, including its bark having the ability to treat diarrhoea and abdominal pain (Du Preez, 2012), as well as to treat tooth decay and toothaches (Chinsembu, Hijarunguru and Mbangu, 2015). In Bangladesh it has been stated that other species' milk juices, *F. benghalensis* and *F. racemosa* are used to treat dysentery and diarrhoea respectively (Kadir, Sayeed and Mia, 2013). There are other reports conducted in Iran, stating that the plant *F. carica* is not only used to treat diarrhoea, but is also used for respiratory problems, skin inflammation and also reduces cholesterol and blood triacylglycerides (Bahmani, Zargarani and Rafieian-Kopaei, 2014). All the traditional uses of different species of *Ficus* mentioned above agree with the use of *F. sycomorosa* in this study as a medicinal plant as mentioned in the results.

Located in different parts of Africa, *G. senegalensis* is among the plants used by traditional healers in Limpopo, South Africa to treat diarrhoea. An infusion is made with the leaves of the plant and administered thrice daily until diarrhoea subsides

(Semenya and Maroyi, 2012). This shrub from the family Celastraceae can also be used to treat skin rashes, oral candidiasis and herpes (Chinsemu, Hijarunguru and Mbangu, 2015).

H. procumbens, commonly known as Devil's claw from the family Pedaliaceae, is a medicinal plant known to be used not only in Namibia but other parts of the world, including Botswana, Angola and South Africa (Mncwangi *et al.*, 2012). *H. procumbens* tuberous roots are used in different ways to treat different ailments including stomach pain, postpartum pain, fevers, and as an ointment in Namibia by the Topnaar people. Cold infusions made with the tuberous roots are used to treat coughs, diarrhoea, constipation, syphilis and gonorrhoea by the Herero (Mncwangi *et al.*, 2012). This too is in agreement with the results from the study as the Herero people are using the roots for treatment.

Belonging to the family of Moringaceae, *M. oleifera* is a plant rich in nutrition and has medicinal properties (Gopalakrishnan, Doriya and Kumar, 2016). *M. oleifera* leaves are the most used traditional medicine, and the barks may also be used to treat stomach pain, ulcers and to assist in digestion (Leone *et al.*, 2015). This plant is said to act as an anticancer, antimicrobial, antioxidant, antidiabetic and anti-atherosclerotic agent (Gopalakrishnan, Doriya and Kumar, 2016). Both the leaves and the pods have been reported to treat diarrhoea. This information is in agreement with the results obtained from this study as the leaves were reported to have been used to treat stomach pain.

Species belonging to the genus *Tephrosia* belong to the family Fabaceae. Information on *T. burchellii* is very scarce, but reports on other species belonging to this genus are recorded. One such species is *T. purpurea*, a shrub found in Pakistan whose roots are used in a decoction to treat diarrhoea and colic pain (Qureshi, 2012), while in India a decoction of the roots is made with honey and administered orally (Jain, Singh and Singh, 2011). This information is in agreement with the results from the study as decoctions of *T. burchellii* roots are also used to treat diarrhoea.

Terminalia sericea, a common shrub belonging to the family Combretaceae, has multiple uses ranging from land improvements to medicinal uses (Amri, 2011). As a medicinal plant, *T. sericea* roots are used to treat diarrhoea, pneumonia, colic and bilharzia, while the leaves are used for stomach disorders (Amri, 2011). This report together with a study conducted in Botswana document the use of the roots to treat diarrhoea and stomach problems (Mukanganyama *et al.*, 2011). This information is in agreement with the results from the study as the roots are also used to treat stomach pains. However it differs in the way the leaves are used as in this study, the leaves are used to treat swollen legs and not for stomach disorders.

X. caffra, commonly known as large sour plum, from the family Ximeniaceae is known to be distributed in Africa from East Africa to countries in southern Africa including Botswana and Namibia (Nair *et al.*, 2013; Maroyi, 2016). As in the study conducted, the roots of this plant are used in the treatment of dysentery and diarrhoea, and in addition to that, the roots can also be used in combination with the leaves to treat abdominal pain and bilharzia. (Nair *et al.*, 2013). Furthermore, Nair *et al.* (2013), states

that the powdered dried leaves are taken orally for fever and infertility, and powdered roots are applied on wounds and infections to aid healing. Additionally the leaves are used in northern Maputaland region of KwaZulu-Natal in South Africa to treat venereal diseases (Nair *et al.*, 2013), whereas in Zambia, the roots are used (Chinsembu, 2016).

Not only are these plants known for their medicinal purposes but they also contain edible fruits and nuts and they also have an oil commonly known as Ximenia oil used as a moisturiser and several products have been produced from it (Nair *et al.*, 2013; Maroyi, 2016). In Namibia, *X. caffra* and *X. americana* are commercially important for rural communities and sold plants generated an income of over \$110 000 in 2008 (Maroyi, 2016).

5.2. Antimicrobial activity of crude extracts

As seen in the results, the plant extracts had antimicrobial activity against both gram-positive and gram-negative bacteria, however the inhibitory effects differed for both aqueous and organic extracts. With some of them having activity, it can be said that they have the potential to be used as a treatment method against GITI.

Some plants had no activity or a low inhibitory effect for all the microorganisms at 100 mg/mL. These included *D. tomentosa*, *S. birrea*, *S. araliaceae*, *M. sylvestris*, *I. hochstetteri*, *D. condylocarpon*, *S. tenuinervis*, *P. capensis*, and *M. cerviana*. Reports on the traditional medicinal use of *M. sylvestris* and *I. hochstetteri* is not available in literature and therefore this will be the first to the best knowledge of the author.

There is insufficient literature on the medicinal use of most of these plants. **Table 10** describes their use, including the plant part used. Information shown in the table about some of the plants, such as *D. tomentosa*, and *S. birrea*, are in accord with the use of the plant parts used in the study to treat symptoms related to GITI in Namibia. Several studies, including those mentioned in **Table 10** have reported the use of the stem bark of *S. birrea* as treatment for diarrhoea. As the results revealed a small zone of inhibition on *B. subtilis* and *E. coli* only; this could mean that the leaves do not possess the properties that the barks do have, and so are not as effective against organisms causing symptoms related to GITI. *P. capensis* roots do possess antimicrobial activity as they are also used to treat TB-like symptoms (**Table 10**). According to the results, all these plants have a broad spectrum of activity as they were able to inhibit the growth of both gram-positive and gram-negative microorganisms.

M. sylvestris aqueous extract revealed antimicrobial activity against *B. subtilis* and *S. aureus*, yet did not inhibit the growth of the gram negative microbes used in the study. This could mean that this plant has a narrow spectrum of activity, and is only effective against gram-positive microorganisms. Seeing as it only had activity against these organisms, it means that it might not be able to improve symptoms brought on by Enterobacteriaceae and kill these microbes. The *I. hochstetteri* aqueous extract on the other hand had antimicrobial activity against both gram-positive and gram-negative microbes, revealing that it has a broad spectrum of activity.

Table 11: Traditional uses of medicinal plants

Plant	Family	Use	Part	Reference
<i>D. tomentosa</i>	Asteraceae	Malaria, cough, postnatal care, diarrhoea	Whole plant	(Jansen <i>et al.</i> , 2012; Urso <i>et al.</i> , 2016)
<i>S. birrea</i>	Anacardiaceae	Diabetes, diarrhoea, dysentery, malaria	Leaves, bark	(Cordell, 2015; Van Wyk, 2015)
<i>S. araliaceae</i>	Apiaceae	Headache	Roots and leaves	(Chinsebu, Hjarunguru and Mbang, 2015)
<i>D. condylocarpon</i>	Apocynaceae	Diarrhoea	Leaves	(Chinsebu, Hjarunguru and Mbang, 2015)
<i>S. tenuinervis</i>	Anacardiaceae	Menorrhagia	Leaves	(Maroyi, 2013)
<i>P. capensis</i>	Chrysobalanaceae	TB-like symptoms	Root	(York, De Wet and Van Vuuren, 2011)
<i>M. cerviana</i>	Molluginaceae	Fever, burning urination, sexually transmitted diseases	Roots	(Malik <i>et al.</i> , 2015)

Choosing the extracts with the highest inhibitory effects on each microbe, the extracts from the plants *A. erioloba*, *Aloe sp.*, *F. sycomorus*, *G. senegalensis*, *H. procumbens*, *M. oleifera*, *T. burchellii*, *T. sericea* and *X. caffra*, are discussed in detail.

5.2.1. *A. erioloba*

The aqueous extract of *A. erioloba* had activity against the gram-positive bacteria, and *E. coli* and no activity against *S. sonnei*. The organic extracts, however, had inhibitory effects against all the microorganisms except *E. coli*, with the highest one being against *B. subtilis*, with a zone of inhibition of 8 ± 0.3 mm and a MIC of 100 mg/mL. This

means that it is very susceptible to *A. erioloba*. An experiment done in Botswana tested the antimicrobial activity of *A. erioloba* on *B. subtilis* and *S. aureus*, and found that the crude ethanol leaf extracts had activity, with zones of inhibition of 1.0 ± 0.00 mm and 1.5 ± 0.00 mm respectively, and no activity against *E. coli* (Mukanganyama *et al.*, 2011). Results obtained are in agreement with those from the study as the organic extracts revealed activity of *A. erioloba* against *S. aureus* and *B. subtilis* but none against *E. coli*.

5.2.2. *Aloe sp.*

The genus *Aloe* has many species that have been widely studied, revealing their medicinal and cosmetic uses. From the study, *Aloe sp.* was not seen to have antimicrobial activity when the aqueous extract was tested on the microbes, but the organic extracts were able to inhibit the growth of the organisms, showing zones of inhibition. This could be because the aqueous extraction method used for this study differed from what is used in traditional settings. In traditional settings, the leaves are boiled and the water from that is taken orally (**Table 5**), whereas in this study, cold water was used. This was because one standard method was to be used for all the plants. It is also seen in the results that *Aloe sp.* has antimicrobial activity against both gram-positive and gram-negative bacteria, which has also been mentioned by Cellini *et al.* (2014). The organic extract of *Aloe sp.* leaves was found to have the highest inhibitory effect against *B. subtilis* (MIC = 100 mg/mL). In addition to having antimicrobial activities against the bacteria tested in this study, *Aloe vera* inner gel have been seen to have antimicrobial effects against susceptible and resistant strains of *H. pylori*, with MIC of the lab strain being 100 mg/mL (Cellini *et al.*, 2014).

5.2.3. *F. sycomorus*

F. sycomorus had antimicrobial activity against *B. subtilis* and *S. aureus* and had a MIC of 100 mg/mL against *B. subtilis*. This could mean that it has narrow activity and is not effective against gram-negative bacteria. Literature documents the antimicrobial activity of the leaf extracts of *F. sycomorus*, but not much is reported on the bark's use against the causative agents of GITI. One activity of the bark reported is its hypoglycaemic effects (Adoum, Micheal and Mohammad, 2012).

A study in Burkina Faso shows that the leaves possess an antimicrobial activity against *E. coli* (MIC of 0.63 mg/mL) and *S. aureus* (MIC of 0.31 mg/mL) (Ramde-tiendrebeogo *et al.*, 2012). Another species, *F. craterostoma*, is known to have antidiarrhoeal properties and has activity against *E. coli*, *S. aureus* and *S. flexneri* (Madikizela *et al.*, 2012). This, however, is not the case in this study as no activity was seen against *E. coli* and *S. sonnei*. It can be said that the leaves would be better at inhibiting the growth of microbes responsible for GITI than the bark as they have shown to have a broad spectrum of activity.

The phytochemical constituents suggest that these plant parts have antimicrobial activity. The phytochemical constituents of the leaf extracts were found to be phenolics, flavonoids, flavonols and tannins (Ramde-tiendrebeogo *et al.*, 2012), while the bark possesses saponins, alkaloids, tannins, glycosides, flavonoids and resins (Adoum, Micheal and Mohammad, 2012). The tannins, glycosides, phenolics and alkaloids are all grouped as chemicals that have antimicrobial activity. This explains why *F. sycomorus* had activity against *B. subtilis* and *S. aureus* in the study. Lack of

activity against *E. coli* and *S. sonnei* could have been due to many factors, including the growth conditions of the bacteria and errors made in subjecting the bacteria to the treatment.

5.2.4. *G senegalensis*

G. senegalensis was observed to have antimicrobial activity against three of the microbes in the study except for *S. sonnei*. The highest activity was seen against *E. coli* with the aqueous extract having a MIC of 100 mg/mL. This could mean that it is more effective against gram-negative organisms. This shows that it has a wide spectrum of activity as it was able to inhibit the growth of both gram-positive and gram-negative bacteria. Its leaf extract inhibitory effect against *E. coli* and *S. aureus* has been reported in a study by Ahmed *et al.* (2013).

5.2.5. *H. procumbens*

From the results the aqueous extract only had an inhibitory effect against *E. coli*, and *B. subtilis*. Since infections with these organisms causes diarrhoea amongst other symptoms related to GITI, the results show that *H. procumbens* has antimicrobial activity and has the ability to treat diarrhoea. It could be that the organic extraction method used was not good enough to produce a product that could be effective against the microorganisms, and so no activity was seen. It can be postulated that the phytochemical constituents of the roots, iridoids, harpagoquinones, amino acids, flavonoids and carbohydrates are responsible for the antimicrobial activity observed (Mncwangi *et al.*, 2012).

5.2.6. *M. oleifera*

The results show that the aqueous extract of *M. oleifera* was only active against *B. subtilis* at 100 mg/mL concentration. This is contradictory to what has been reported in other studies. The leaf acetone extract of *M. oleifera* at 5 mg/mL concentration showed antibacterial activity against *E. coli*, *S. aureus* and other microorganisms. In this study, the organic extraction solvent used could have been responsible for the lack of activity seen against any of the microorganisms (Moyo, Masika and Muchenje, 2012). Studies have shown the presence of numerous types of phytochemicals in *M. oleifera* leaves, which include nitrogen-containing phenolic glycosides, niazirin and glycosides (Mishra *et al.*, 2011; Sahakitpichan *et al.*, 2011). Also found in the leaves are alkaloids, flavonoids, saponins, tannins, oxalates, phylates, glucosinolates and isothiocyanates (Leone *et al.*, 2015). This may explain why they have many medicinal properties as these phytochemicals are known to have antibacterial properties amongst other properties.

5.2.7. *T. burchellii*

An experiment conducted on *T. purpurea* showed that it had a high inhibitory effect against *Propionibacterium acne*, with an MIC of 0.049 mg/mL (Saranraj and Sivasakthi, 2014). Another species, *T. cinerea* leaves are used to treat GITI in Mexico, and a study showed that it has inhibitory effects against *S. aureus* (Sharma *et al.*, 2016). Sharma *et al.* (2016) has also reported the presence of flavonoids and quinones in *T. cinerea* and *T. purpurea* root extracts has been found to possess flavonoids, tannins, and antioxidant activities (Nile and Khobragade, 2011; Palbag, Dey and Singh, 2014). In addition *T. purpurea* extracts have significant antiulcer property (Palbag, Dey and Singh, 2014).

Reports on other species, including *T. calophylla* and *T. vogelii*, show that these plants also possess antimicrobial (Touqeer, Saeed and Ajaib, 2013). *T. vogelii* leaf extracts have also been reported to have significant activity against *S. aureus* and *E. coli* and different parts of the plant have also been found to possess antiulcer activity, and contains sesquiterpenes, lignin and rotenoid as phytochemicals (Touqeer, Saeed and Ajaib, 2013). Reports on *T. burchellii*'s antimicrobial activity and phytochemical constituents are not available, but as species from the same genus have been extensively studied it is possible that the activity of *T. burchellii* seen against all tested microorganisms is not coincidental but may be due to the same phytochemicals found in *T. cinerea* and *T. purpurea* as they are known to possess antimicrobial properties.

5.2.8. *T. sericea*

In this study, *T. sericea*, had antimicrobial activity against all the microorganisms, with *B. subtilis*, *S. sonnei* and *S. aureus* being susceptible to all the plant parts (leaves, roots and bark) while *E. coli* was only susceptible to the root extract. The root extract had a MIC of 0.1 mg/mL and 1 mg/mL against *S. aureus* and *S. sonnei* respectively, and 100 mg/mL against *E. coli*. The results show that the root extracts of *T. sericea* are highly active against *S. aureus* and *S. sonnei* but weakly active against *E. coli*.

Studies have shown that the leaves of this plant are also used to treat stomach disorders, validating the broad spectrum inhibitory effect of this plant seen in the results of this study. The inhibitory effect however is lower than that of roots. Studies have shown that *T. sericea* exhibited a broad-spectrum of activity and had antimicrobial activity against *S. aureus* with a MIC of 1.60 mg/mL (Mabona *et al.*, 2013). *T. sericea*'s

antimicrobial activity could be due to tannins and punicalagin (Ndhlala *et al.*, 2013; Chinsembu, 2016).

5.2.9. *X. caffra*

From the study, the roots of *X. caffra* had high antimicrobial activity against *E. coli*, *S. sonnei* and *S. aureus*, which is seen in the results by the zones of inhibition observed. As they had the highest antimicrobial activity, the MIC was determined for each plant and found to be 1 mg/mL for *X. caffra* against all three microorganisms. Several studies have revealed that organic root extracts of *X. caffra* on microbes known to cause diarrhoea were active with MICs of 0.156 mg/mL against *Shigella flexneri* and *V. cholera*, and 0.195 mg/mL against *B. subtilis* and *S. aureus* (Nair *et al.*, 2013) This demonstrates the high activity of this plant against bacteria but also validates its use in traditional settings as a treatment against symptoms related to GITI. Maroyi (2016) reports that *X. caffra* roots have tannins, flavonoids, gallotannins and phenolics, which are known to be responsible for antibacterial activity. This could be the reason why the results showed a high antimicrobial activity of *X. caffra* tested against *E. coli*, *S. sonnei* and *S. aureus* in this study.

5.3. Antimicrobial activity of fractionated samples

Fractionated samples were produced for *X. caffra* and *T. sericea* root extracts. For *X. caffra*, small zones of inhibition were seen at 100% ethyl acetate when tested against *S. aureus* and *S. sonnei* and at 20% methanol in DCM against *E. coli*. On the other hand methanol (100%) fraction from *X. caffra* root extract produced the highest antimicrobial activity against *E. coli* (MIC = 100 mg/mL) and *S. aureus* (MIC = 0.1

mg/mL) while 10% acetone in DCM produced the highest antimicrobial activity against *S. sonnei* with a minimum inhibitory concentration of 0.01 mg/mL. This shows that against *E. coli*, *X. caffra* has weak activity, while against *S. aureus* and *S. sonnei* it has very high activity. As mentioned above, *X. caffra* roots possess tannins, flavonoids, gallotannin and phenolics, which could be responsible for activity. In the same way, the fraction with the highest activity most likely possesses these phytochemicals.

T. sericea root extracts had no activity at 100% methanol fraction when tested against *E. coli* and *S. aureus*, and also no activity was seen against *E. coli* at the 50% acetone in DCM fraction. When tested against *S. sonnei* the lowest zone of inhibition was seen at the 50% acetone in DCM. Just like in the case of *X. caffra*, *T. sericea* root extracts were seen to have weak antimicrobial activity against *E. coli*, with MIC of 100 mg/mL at 100% ethyl acetate fraction. High antimicrobial activity was seen against *S. aureus* and *S. sonnei* (MIC = 0.01 mg/mL) for the fractions 20% methanol in DCM and 10% acetone in DCM respectively. This could be because of tannins and punicalagin in the fractions.

CHAPTER 6: CONCLUSION

Firstly, this study was meant to document medicinal plants used to treat symptoms related to gastrointestinal infections in Namibia. This was done through an ethnobotanical survey and a number of plants were collected and documented. In conclusion, the objective was met as a list of eighteen plants were documented, clearly showing how they are being used in traditional settings. This information is not well documented and this study has aided in this. To validate the use of these plants, antimicrobial tests were performed on them against microorganisms that cause infection in the gastro-intestinal tract. The methods used are as described above in Chapter 3.

As mentioned, the study has documented eighteen medicinal plants used in traditional settings to treat symptoms that are associated with GITI in Namibia. These have been validated with antimicrobial activity ranging from low to high. *X. caffra* and *T. sericea* were the plants with the highest antimicrobial activity and a broad spectrum of activity against gastroenteritis causative agents. Some of the tested plants have been reported in literature, while some of them have very little information available, such as *T. burchellii* and others not being documented (*I. hochstetteri* and *M. sylvestris*) Other species belonging to the genus *Tephrosia* have been documented and their activity and phytochemical activity are known. *T. burchellii*, on the other hand has not been well documented as only its use in traditional settings has been documented. It was therefore necessary to perform antimicrobial tests so as to validate its use in traditional settings. In conclusion, medicinal plants used to treat symptoms related to GITI were documented and their antimicrobial activity against the causative agents determined.

CHAPTER 7: RECOMMENDATIONS

In this study, certain outcomes were obtained, namely, the documentation of medicinal plants used to treat symptoms associated with GITI and to determine the antimicrobial activity of these plants. Having done all this during the study, some shortcomings were encountered. First of all, during the ethnobotanical survey, information on the plant dosages were not all stated as some of the respondents did not have this information. Secondly, the cytotoxicity of the plants were not determined. Lastly, the active compounds and the chemical structure of these active compounds were not determined.

Therefore, it is recommended that the dosage of the plants used be known as this will determine the right amount to administer without causing any harm to the patient. This can be done by choosing the concentrations of the extract which are able to treat symptoms related to GITI based on a person's age and gender. As cellular safety was not determined by this study, further research on the cytotoxicity should also be done. Furthermore, research should be conducted to determine the phytochemical constituents of the plants, and to determine the chemical structure of the active compounds in the crude extracts. Furthermore, the active principal of the highest active fractions of *X. caffra* and the root extracts of *T. sericea* should be determined. Progression in research work towards developing an alternative treatment available to all will therefore be possible.

CHAPTER 8: REFERENCES

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APPENDICES

APPENDIX A

ETHNOBOTANY, PHYTOCHEMISTRY AND BIOACTIVITY OF MEDICINAL PLANTS USED TO TREAT PEPTIC ULCER DISEASE IN NAMIBIA

Lisanza T. Mulyangote, Student number: 200907531

MSc Ethno-botanical research questionnaire

Informant's details:

Questionnaire number: _____ Date _____
Gender: _____ Age: _____
Duration of practice: _____ Village name: _____
GPS Location: _____ Constituency: _____
Region: _____ Do you have an apprentice? _____
Age of apprentice: _____ Gender of apprentice _____
Knowledge of medicinal plant use _____

Disease details:

Local name of the disease and language _____
Symptoms of the disease _____
Complications of the disease _____
Gender of patients _____
Characteristics about the patient _____
Length of treatment _____

Plant Description:

Local name of the plant _____
Voucher number _____
Species _____ Perennial/Annual
Habit (e.g. shrub) _____ Height _____ (m)
Habitat (where is it collected & growing?) _____
How is the plant harvested? _____

Is a permit required to collect the plant? _____

Occurrence (e.g. common) _____

Flower/inflorescence (colour, shape, size, smell, texture) _____

Leaves (simple/compound, margin, shape, colour, texture, stipules) _____

Stem and bark (colour, texture, habit e.g. erect stem with yellow papery peeling bark – presence or absence and smell of sap or latex – e.g. milky latex with unpleasant smell) _____

Fruits and seed (presence/absence, shape, colour, edibility and taste if edible, single or clustered, maturity) _____

Roots and underground organs (shape, texture, colour, size) _____

Other (e.g. uses, ecology) _____

Plant part used (Roots, leaves, barks, whole plant): _____

Mode of preparation (infusion, decoction or sieved; length of extraction, how much water is used?): _____

Dosage:

- How often is the herb given to patient and for how long? _____

- How is it taken? (orally or mixed in food) _____

- Is it taken using one plant or a combination of plants? If taken as combination therapy, how many plants are used and what are the proportions? _____

What other diseases does the plant treat? _____

APPENDIX B



MINISTRY OF ENVIRONMENT AND TOURISM

RESEARCH/COLLECTING PERMIT

Permit Number 2214/2016

Valid from 1 November 2016 to 31 October 2017

Permission is hereby granted in terms of the Nature Conservation Ordinance 1975 (Ord. 4 of 1975) to:

Name: Prof. K.C. Chinsebu
Address: University of Namibia
Faculty of Science
Private Bag 13301
Windhoek
Namibia

Coworkers: L.T. Mulyangote and Dr. M. Hedimbi

Ethnobotany, phytochemistry and bioactivity of medicinal plants used to treat peptic ulcer disease in Namibia in Oshana and Omusati regions, subject to attached conditions.

IMPORTANT: This permit is not valid if altered in any way.




Authorising Officer

IMPORTANT

This permit is subject to the provisions of the Nature Conservation Ordinance, 1975 (Ordinance 4 of 1975) and the regulations promulgated thereunder, and the holder is subject to all such conditions and regulations.

Enquiries: Conservation Scientist, email ija.mahus@met.gov.na
Private Bag 13306, Windhoek, Namibia

APPENDIX C

NBRI: DATA COLLECTION FORM								
Collect.No.....			Date.....					
Collector/s.....			GPS.....					
Locality (please describe properly, not just GPS).....			Grid.....					
.....			Altitude.....					
Aspect.....	Slope.....		Exposure.....					
Photo no's.....	Voucher for.....							
DESCRIPTION OF PLANT Please give as much information as possible								
Species.....			Perennial/Annual					
Habit (e.g.shrub).....			Height.....		cm			
Occurrence (e.g.common).....								
Indigenous names and languages.....								
Flower/Inflorescence (colour,shape,size,smell,texture).....								
Leaves (simple/compound,margin,shape,colour,texture,stipules).....								
Stems and bark (colour,texture,habit - e.g. erect stem with yellow papery peeling bark - presence or absence and smell of sap or latex - e.g. milky latex with unpleasant smell).....								
Fruits and seed (presence/absence,shape,colour,edibility and taste if edible, single or clustered,maturity).....								
Roots and underground organs (shape,texture,colour,size).....								
Other (e.g. uses,ecology).....								
Habitat			Vegetation	Substrate	Soil type	Lithology	Moisture	Biotic effect
cliff face	plain	lake/pond	desert	soil	sand		well-drained	road/rail side
mountain/hillside	plateau	marsh/swamp/wetland	shrubland open/closed	stone/rocky soil	sandy loam	mica schist	moist/damp	cultivated land
mountain/hill peak	valley	river/stream	forest	gravel	loam	dolomite	seasonally waterlogged	abandoned land
tallush/reef	donga/gully/ditch	river/stream bank	grassland	bare rock	loamy clay	granite	permanently waterlogged	heavily grazed
					gravel			
dune slope	depression/pan	seepage area	karoo	water	clay	lime	free standing water	garden
dune crest	dam	waterfall/rapids	thornbush savanna	roots	calcrete	quartzite	running water	plantation
intardunal street	dry river/stream bed	unknown		termit mound	black turf	other	mist/fog	recently burnt
ravine/kloof/gorge	foodplain			other	humus rich		tidal	disturbed - other
ridge	estuary/leagoon				salt/brack			other